









# PHYTOPATHOLOGY

---

VOLUME XII, 1922.



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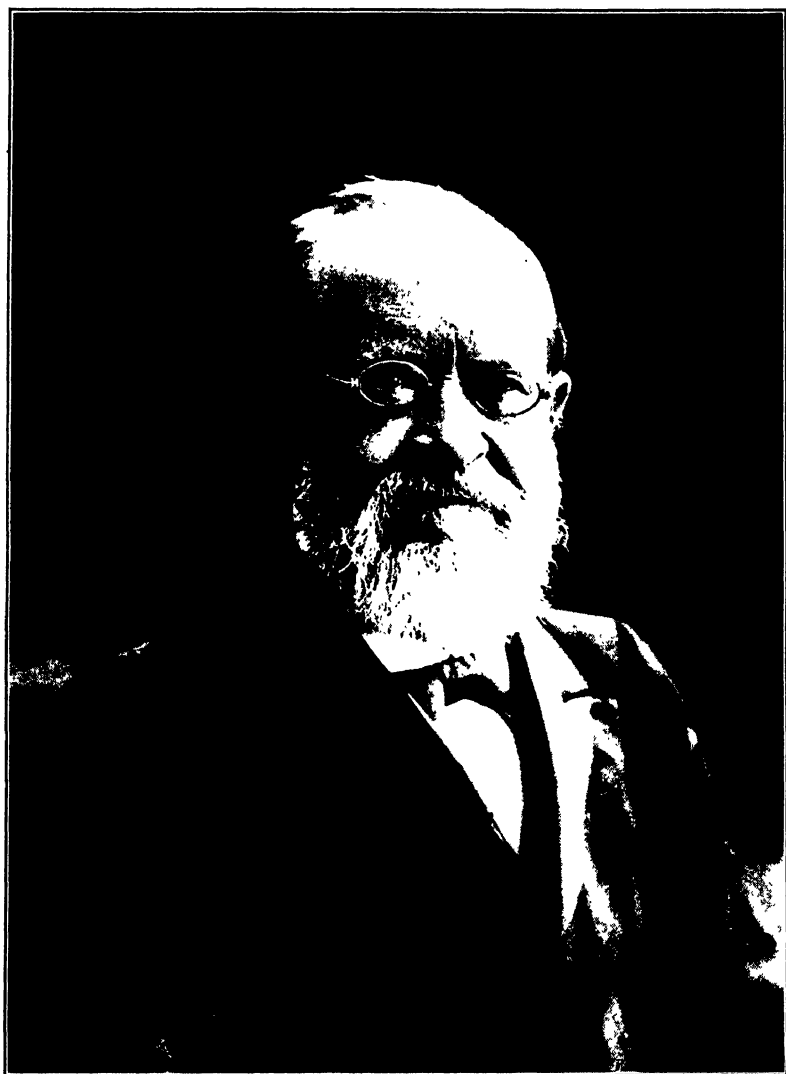
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E. ROSTRUP

# PHYTOPATHOLOGY

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FREDERIK GEORG EMIL ROSTRUP

1831-1907

C. L. SHEAR<sup>1</sup>

WITH PORTRAIT, PLATE I

On a pleasant July afternoon in 1905, the writer took the train from Copenhagen for Helsingor, Denmark. The purpose of this pilgrimage was not that of the ordinary tourist to see the Castle of Helsingor or Hamlet's Tomb, but to pay a visit to the distinguished Danish pathologist, Dr. E. Rostrup, who was spending his vacation at his country home a few miles from Helsingor.

Upon my arrival I found a most genial old gentleman who cordially welcomed me, and after tea was served and a period of social intercourse with his wife and daughter we spent the remainder of the afternoon in discussing pathological and mycological matters and examining such cases of plant diseases as his garden and grounds furnished. The accompanying portrait is from a photograph kindly presented me at the time, and evidently taken not long before. I was so impressed by his simplicity and kindness as well as his profound knowledge of plant diseases and fungi, that it was with deep regret and the feeling that I had lost a friend as well as colleague, that I received from his daughter less than two years later the announcement of his death.

Frederik Georg Emil Rostrup, usually written simply E. Rostrup by himself, was born January 28, 1831, at Stensgaard on the island of Lolland and died January 16, 1907. He was born and reared in the country and at a very early age showed a deep interest in nature. This appears to have been largely due to his mother's influence. When only thirteen he began the systematic collection and study of the plants,

<sup>1</sup> Much of the information in this sketch is from F. Kölpin Rayn's biographical sketch in *Ber. Deutsch. Bot. Gesellsch.* 26: 47-55, 1908, and J. Lind's introduction to his volume on "Danish Fungi as represented in the Herbarium of E. Rostrup," p. 1-9, 1913.

birds and insects of the neighborhood. A few years later he recorded a series of phænological observations on the blooming period of the native plants. He was very fond of all forms of outdoor exercises and enjoyed hunting especially, on account of the opportunity it offered of studying animals. Although he had no special instruction in science at this time he read diligently all the available works on natural history and improved every opportunity to increase his knowledge by field observations. After acting for several years as clerk in his father's office he went to Copenhagen when nineteen years old and took up the study of mathematics and natural history in the polytechnic school and university. Here he came in contact with Oersted, the botanist, and other scientists. He also became a member of a scientific seminar called "The Cell" and took an active part in the presentation and discussion of papers. In 1857, he ended his scientific studies in Copenhagen and became a teacher of science and mathematics in the training school at Skaarup in southern Funen. Here he spent twenty-five years. About this time he also began to take an active interest in mycology and pathology and collected and studied at first, as usual, the most conspicuous forms of fungi, such as agarics and polypores, and later all the species of any group he could find. He was fortunate in having access to a very good scientific library, but was not satisfied with the descriptions he found in the literature of that time. He therefore began keeping a notebook and diary, and recording his observations on the various fungi and diseases found. This was continued for more than 46 years. His deep and enthusiastic interest in science and his great devotion to study and investigation must have added interest to his teaching and naturally resulted in many of his students eventually becoming investigators. His first published scientific work was a paper on Plant Geography in 1858. In 1860 he prepared a guide to the Danish Flora, which has passed through ten editions. In his teaching and study of general botany and of all groups of plants, he acquired that familiarity with the higher plants which is of fundamental importance as a basis for the most successful prosecution of certain phases of mycological and pathological work, especially where hosts relations are involved. In 1866 he published his first contribution to mycology and pathology, entitled, "Cultural Studies of *Sclerotia*." From this time on he devoted more and more attention to pathology and other phases of economic botany. In fact he was always especially interested in problems of practical interest to agriculture and horticulture. He said, "I have always, if possible, tried to combine my scientific researches with useful objects." In his early pathological work he devoted much attention to diseases of forest trees. In 1883 he left Skaarup and returned to Copenhagen where the remainder

of his life was spent. He first received an appointment as Docent and later became lecturer, and in 1902 was made professor in the Royal Veterinary and Agricultural College. His practical knowledge of agriculture and of country life and conditions were of great advantage to him in his pathological studies and their application. People of all classes came to consult him in regard to practical agricultural and pathological problems and he delighted in giving them all the assistance possible.

He published over 100 important papers besides numerous briefer contributions covering many phases of botanical work ranging from paleobotany to botanical folklore. He accumulated an herbarium of about 30,000 specimens, most of which were collected by himself and his students. He was the recipient of many honors during his life, including Danish, Norwegian and Swedish decorations and the degree of Doctor of Philosophy from the University of Copenhagen. His latest and most important volume was on plant pathology, entitled in Danish "Plantepatologi." This appeared in 1902 when he was 72 years of age.

In commemoration of his life and service a fine monument has been erected in the grounds of the Agricultural College in Copenhagen. A greater tribute to his memory, however, is the affection with which he is remembered by all who came into intimate relations with him, either as students or coworkers. The interest and enthusiasm for mycology and pathology which he inspired in his students, many of whom are now carrying on important work in these fields as well as in other lines of research, will be passed on to others in an ever widening circle, thus perpetuating his work and influence.

# INFECTION CAPABILITIES OF CROWN RUST OF OATS

G. R. HOERNER<sup>1</sup>

## INTRODUCTION

Crown rust of oats is practically coextensive with the culture of the host crop. It has generally been considered one of the most destructive of any of the American leaf rusts of cereals. Foreign uredinologists as a whole do not consider it of primary importance. On the North American continent, the fact that it is commonly associated with the stem rust makes a fair estimate of the losses occasioned difficult. In the southern and western United States especially, however, there is no doubt that it is oftentimes a limiting factor in oat production.

The crown rust of oats has long been of scientific interest. A bibliographical digest is in the course of preparation for publication at some future date.

The purpose of the inoculation experiments presented in this paper was to determine not only the grass and cereal hosts in general but whether these hosts varied for the individual specimens of rust collected in different localities of the United States where particular varieties of oats were commonly grown and where the grasses found in those localities might be limited.

## METHODS AND MATERIALS

In the inoculation work on cereals and grasses, the results of which are presented in another part of this paper, the methods and materials described by Stakman and Piemeisel<sup>2</sup> in their work with stem rust were adopted.

The grass seed from which seedlings used in the inoculation work herein recorded were grown was obtained from the Minnesota and Montana Seed Laboratories. The seed of wheat, barley, rye, and some of the oat varieties was secured from the Agronomy Division, University of Minnesota; the club wheat from the Washington Experiment Station at Pullman; various oat varieties were secured from the Alabama, California, Iowa, and Virginia experiment stations.

<sup>1</sup> Investigations carried on while a graduate student at the University of Minnesota. 1916-1918.

<sup>2</sup> Stakman, E. C. and Piemeisel, F. J. *Biologic forms of Puccinia graminis on cereals and grasses.* Jour. Agric. Research, 10: 429-496. Pl. 53-59. 1917.

## GRASS AND CEREAL SEEDLING INOCULATIONS

In the following tables the results of inoculations are indicated by a fraction. The denominator indicates the total number of leaves inoculated. The numerator indicates the number of leaves that became infected. The number of leaves that became flecked but did not produce uredinia is indicated by the figure separated from the fraction by the semicolon.

In all subsequent tables *Avena sativa* indicates Ligowa oats, Minn. 281; *Hordeum vulgare*—Manchuria barley, Minn. 105; *Secale cereale*—Swedish rye, Minn. 2; *Triticum compactum*—Brown Gloria club wheat; *Triticum vulgare*—Haynes bluestem wheat, Minn. 169.

TABLE 1—Summarized results of inoculations with urediniospores of various specimens of *Puccinia coronata* Cda.

Plant inoculated	Place of collection of rust and results of inoculations			
	Tallulah, Louisiana		St. Paul, Minnesota	
Agropyron caninum (L.) Beauv.	$\frac{0}{23}$		$\frac{1}{26}$	Minute uredinia
Agropyron cristatum J. Gaert.	$\frac{0}{44};^{10}$	Flecks distinct	$\frac{0}{37};^{12}$	Flecks fairly distinct
Agropyron desertorum Schult.	$\frac{1}{44};^1$	Minute uredinia flecks indistinct	$\frac{1}{39};^7$	Minute uredinia: flecks distinct
Agropyron elongatum Host.	$\frac{0}{8};^2$	Flecks indistinct	$\frac{0}{10};^1$	Flecks indistinct
Agropyron imbricatum Roem. and Schult.	$\frac{0}{25};^3$	do.	$\frac{0}{13};^2$	do.
Agropyron intermedium Beauv.	$\frac{0}{37};^3$	do.	$\frac{2}{17};^9$	Minute uredinia: flecks indistinct
Agropyron repens (L.) Beauv.	$\frac{0}{37}$	$\frac{0}{40}^a$	$\frac{0}{12}$	
Agropyron smithii Rydb.	$\frac{0}{9};^7$	Flecks distinct	$\frac{0}{38};^3$	Extensive dead areas
Agropyron tenerum Vasey	$\frac{0}{7}$		$\frac{0}{16}$	

<i>Agrostis alba</i> L.	$\frac{0}{27}$		$\frac{0}{532}$	*
<i>Agrostis canina</i> L.	$\frac{0}{3}$		$\frac{0}{10}$	
<i>Agrostis stolonifera</i> Vasey	$\frac{0}{30}$		$\frac{0}{35}$	
<i>Alopecurus geniculatus</i> L.	$\frac{0}{51},^{36}$	Flecks distinct	$\frac{164}{569},^8$	* Moderate uredinia; distinct flecks
<i>Alopecurus pratensis</i> L.	$\frac{0}{15},^3$	Flecks indistinct	$\frac{3}{166},^{20}$	$\frac{0}{10}$ b * Minute uredinia; distinct whitened areas
<i>Andropogon furcatus</i> Muhl.	$\frac{0}{4}$		$\frac{0}{13}$	
<i>Anthoxanthum odoratum</i> L.	$\frac{1}{41},^{26}$	Moderate uredinia; flecks distinct	$\frac{7}{61},^{42}$	Moderate uredinia; distinct flecks
<i>Arrhenatherum elatius</i> (L.) Beauv.	$\frac{0}{33}$	$\frac{0}{16},^{3a}$	Flecks indistinct	$\frac{1}{72}$ * Minute uredinia
<i>Avena fatua</i> L.		$\frac{9}{9}$ a	Heavy a	$\frac{30}{42}$ $\frac{9}{9}$ b * Moderate uredinia Heavy b
<i>Avena sativa</i> L.	$\frac{739}{771},^1$	$\frac{135}{150}$ a	Heavy; pigment Heavy a	$\frac{741}{902},^3$ $\frac{128}{155}$ b * Heavy; pigment; telia Heavy b
<i>Avena sterilis</i> L.	$\frac{27}{29}$	$\frac{11}{13}$ a	Uredinia scattered; heavily infected leaves yellowed Heavy a	$\frac{21}{21}$ $\frac{5}{7}$ b Minute uredinia; large white dead areas; telia pigment; some leaves heavy Heavy b
<i>Bouteloua curtipendula</i> (Michx.) Torr.			$\frac{0}{13}$	
<i>Briza maxima</i> L.	$\frac{0}{7}$		$\frac{0}{5}$	Large areas of leaves killed

<i>Bromus ciliatus</i> L.				$\frac{0}{61};^{17}$	*	
<i>Bromus erectus</i> Huds.	$\frac{0}{11};^4$	$\frac{0}{8}$ a	Flecks distinct	$\frac{1}{45}$	* Minute uredinia	
<i>Bromus inermis</i> Leyss.	$\frac{0}{15};^5$	$\frac{0}{10}$ a	do.	$\frac{1}{52}$	* do.	
<i>Bromus japonicus</i> Thunb.	$\frac{0}{23};^3$	$\frac{1}{15}$ a ;1	Flecks indistinct Minute uredinia a	$\frac{0}{87};^4$	* Flecks indistinct	
<i>Bromus purgans</i> L.				$\frac{0}{23}$	*	
<i>Bromus tectorum</i> L.		$\frac{0}{12}$ a		$\frac{1}{133};^{13}$	$\frac{0}{19}$ b	* Minute uredinia
<i>Calamagrostis canadensis</i> (Michx.) Beauv.	$\frac{0}{3};^2$	Flecks indistinct				
<i>Cynosurus cristatus</i> L.	$\frac{0}{12}$			$\frac{0}{31};^2$	Flecks indistinct	
<i>Dactylis glomerata</i> L.				$\frac{16}{32};^1$	Moderately heavy	
<i>Danthonia spicata</i> (L.) Beauv.	$\frac{0}{22}$			$\frac{0}{23}$		
<i>Elymus canadensis</i> L.	$\frac{0}{7};^4$	$\frac{0}{25}$ a	Flecks indistinct	$\frac{0}{35};^{19}$	$\frac{0}{136}$ b	Flecks distinct b do.
<i>Elymus robustus</i> Scribn. and J. G. Sm.	$\frac{0}{15};^4$	$\frac{0}{11}$ a ;5	Flecks distinct; numerous Flecks indistinct a	$\frac{2}{15};^8$	$\frac{0}{18}$ b	Moderate uredinia; telia
<i>Elymus virginicus</i> L.		$\frac{0}{5}$ a		$\frac{0}{10}$		



<i>Festuca elatior</i> L.	$\frac{0}{4}$		$\frac{0}{83}$	
<i>Festuca heterophylla</i> (Lam.) Hack.	$\frac{0}{16}$		$\frac{0}{242}$ ;15	*Flecks indistinct
<i>Festuca ovina</i> L.	$\frac{0}{26}$		$\frac{2}{58}$ ;6	$\frac{0}{45}$ b Minute uredinia; flecks indistinct
<i>Festuca pratensis</i> Huds.	$\frac{0}{38}$		$\frac{0}{72}$	
<i>Festuca rubra</i> L.	$\frac{0}{24}$		$\frac{1}{126}$ ;38	Minute uredinia; flecks indistinct
<i>Holcus lanatus</i> L.	$\frac{0}{52}$		$\frac{3}{71}$ ;2	$\frac{0}{13}$ b do.
<i>Hordeum jubatum</i> L.		$\frac{0a}{18}$ ;3	Flecks distinct a	$\frac{1}{20}$ ;6 Minute uredinia; flecks distinct
<i>Hordeum pusillum</i> Nutt.	$\frac{0}{25}$ ;22	$\frac{9}{14}$ a	Flecks distinct Moderate uredinia a	$\frac{6}{55}$ ;25 Moderate uredinia; flecks distinct
<i>Hordeum vulgare</i> L.	$\frac{0}{35}$ ;10	$\frac{0a}{10}$ ;9	Flecks distinct a do.	$\frac{3}{28}$ ;6 *Very minute uredinia; flecks distinct
<i>Lolium italicum</i> R. Br.	$\frac{0}{29}$ ;2	$\frac{0}{14}$ a	Flecks indistinct	$\frac{0}{54}$ $\frac{0}{12}$ *
<i>Lolium perenne</i> L.	$\frac{0}{10}$	$\frac{0}{7}$ a		$\frac{0}{45}$ $\frac{0}{8}$ b
<i>Lolium temulentum</i> L.	$\frac{0}{29}$ ;25	$\frac{0}{17}$ a	Flecks distinct	$\frac{9}{69}$ ;27 $\frac{0b}{12}$ ;2 Minute uredinia; dead, colorless areas extensive; very hypersensitive Flecks indistinct b
<i>Phalaris canariensis</i> L.	$\frac{0}{9}$			$\frac{0}{28}$

Phleum pratense L.	$\frac{14}{35};^{20}$	Moderate; distinct flecks		$\frac{16}{30};^{14}$	$\frac{0}{15}$ b	Heavy; distinct flecks
Phragmites communis Trin.	$\frac{0}{12}$					
Poa annua L.	$\frac{0}{38};^{36}$	Flecks distinct		$\frac{0}{37};^{36}$		Flecks distinct
Savastana odorata (L.) Scribn.	$\frac{0}{10}$					
Secale cereale L.	$\frac{0}{33};^9$	$\frac{0}{14}$ a	Flecks distinct a do.	$\frac{0}{64};^{10}$		Flecks distinct
Triticum compactum Host.	$\frac{0}{31};^{15}$	$\frac{0}{17}$ a	Flecks distinct a do.	$\frac{0}{37};^5$		do.
Triticum vulgare Vill.	$\frac{0}{33};^9$	$\frac{0}{10}$ a	Flecks distinct	$\frac{0}{26};^3$		do.
Oat Varieties Alabama, Appler 617	$\frac{12}{13}$	Heavy; pigment		$\frac{6}{12};^6$		Uredinia few, scatter- ed; dead, colorless areas; telia in abundance
Alabama, Burt-Spring strain	$\frac{11}{12}$	do.		$\frac{5}{7}$		Uredinia minute; large, white dead areas; telia
Alabama, Fulghum 313	$\frac{19}{19}$	Heavy; pigment		$\frac{13}{14}$		Heavy; pigment
Alabama, Red Rust Proof-Spring strain	$\frac{16}{17}$	do.		$\frac{10}{16};^1$		Heavy
California, Oklahoma Red Rust Proof	$\frac{13}{15}$	do.		$\frac{9}{9}$		Heavy; telia
California, Texas Red Rust Proof	$\frac{10}{10}$	do.		$\frac{13}{13}$		Heavy

Iowa, 73	$\frac{6}{15};^9$	Uredinia few, scattered; white hypersensitive areas	$\frac{7}{11};^4$	Uredinia few, scattered; white hypersensitive areas
Iowa, 96	$\frac{9}{7};^7$	White hypersensitive areas	$\frac{8}{10};^2$	Uredinia few, scattered; white hypersensitive areas
Iowa, 101 1/2	$\frac{11}{13};^2$	Most leaves heavy; few with scattered uredinia; white hypersensitive areas	$\frac{14}{14}$	Most leaves heavy; few leaves with few uredinia and white hypersensitive areas
Iowa, 102 1/2	$\frac{2}{6};^4$	do.	$\frac{16}{16}$	do.
Iowa, 103 (Minn. 531)	$\frac{26}{26}$	Heavy; pigment	$\frac{28}{28}$	Heavy; telia
Iowa, 115	$\frac{12}{12}$	Heavy	$\frac{7}{7}$	Heavy
Minnesota, Golden Rain 528	$\frac{29}{29}$	do.	$\frac{12}{12}$	Heavy; white areas among uredinia; telia
Minnesota, Joonette 550	$\frac{14}{14}$	Heavy; pigment	$\frac{16}{16}$	Heavy
Minnesota, Red Rust Proof 538	$\frac{16}{16}$	Heavy	$\frac{14}{14}$	do.
Minnesota, Silver Mine 506	$\frac{14}{14}$	Heavy; pigment	$\frac{18}{18}$	do.
Minnesota, Swedish Crown 526	$\frac{24}{24}$	Heavy	$\frac{14}{14}$	Heavy; telia
Minnesota, Victory 514	$\frac{19}{22}$	Heavy; pigment	$\frac{13}{13}$	do.

Minnesota, White Russian	$\frac{26}{26}$	do.	$\frac{12}{12}$	Heavy; pigment
Minnesota, 261	$\frac{21}{21}$	do.	$\frac{20}{22}$	do.
Minnesota, 281	$\frac{23}{23}$	do.	$\frac{27}{27}$	Heavy; telia
Minnesota, 512	$\frac{22}{22}$	do.	$\frac{20}{20}$	Heavy
Virginia, Burt	$\frac{14}{14}$	do.	$\frac{9}{12}$	Heavy; telia
Virginia, Swedish Select	$\frac{13}{13}$	do.	$\frac{7}{14}$	do.
Virginia, Texas Red Rust Proof	$\frac{8}{12}$	Heavy	$\frac{14}{14}$	do.

\* In some of the trials, urediniospores were applied in water suspension by means of an atomizer.

N. B. In order to conserve space and to condense the table, data relative to the specimen of rust collected at Lynchburg, Virginia, is marked with a small letter 'a' placed above the line immediately to the right of all such data wherever it occurs in the table. Data relative to the specimen of rust collected at San Diego, California, is marked in a similar manner with a small letter 'b'.

In addition to the grasses presented in table 1, subsequent inoculations with specimens of crown rust of oats collected in various localities included the following:

Plant Inoculated	Summarized results of inoculations
<i>Bromus villosus</i> Forsk.	$\frac{0}{11}$ ;9
<i>Bromus secalinus</i> L.	$\frac{0}{17}$ ;14
<i>Hystrix patula</i> Moench.	$\frac{4}{30}$ ;26
<i>Poa compressa</i> L.	$\frac{0}{18}$ ;5

Although the lists of hosts and characters of infection are by no means identical for the four specimens of rust used, the differences are, owing in most cases to very weak infection, not very striking nor always consistent.

In the absence of more extensive work and more complete data, therefore, a definite statement as to variations in infection capabilities between these four specimens of rust is purposely avoided. Later work with two of these specimens however partially interprets variations in infection capabilities such as appear in table 1.<sup>1</sup>

It was considered of possible significance to compare the summarized results of urediniospore inoculations with all four of the specimens of *P. coronata* Cda. with those obtained with *P. graminis avenae* Erikss. and Henn. by Stakman and Piemeisel,<sup>2</sup> since the work was carried out under similar environmental conditions and by means of similar technic, using grass seedlings grown in most cases from the same seed lots.

TABLE 2 Comparative results of urediniospore inoculations with *P. coronata* Cda., and *P. graminis avenae* Erikss. and Henn.

Plant Inoculated	Results of Inoculations			
	<i>P. CORONATA</i> Cda.		<i>P. GRAMINIS AVENAE</i> Erikss. and Henn. <sup>2</sup>	
<i>Agropyron caninum</i> (L.) Beauv.	$\frac{1}{49}$	Minute uredinia	$\frac{0}{61}$	
<i>Agropyron cristatum</i> J. Gaert.	$\frac{0}{91}$ ; <sup>22</sup>	Flecks fairly distinct	$\frac{3}{25}$	
<i>Agropyron desertorum</i> Schult.	$\frac{2}{83}$ ; <sup>8</sup>	Minute uredinia; flecks fairly distinct	$\frac{0}{30}$	
<i>Agropyron elongatum</i> Host.	$\frac{0}{18}$ ; <sup>3</sup>	Flecks indistinct	$\frac{0}{25}$	
<i>Agropyron imbricatum</i> Roem. and Schult.	$\frac{0}{38}$ ; <sup>5</sup>	do.	$\frac{0}{20}$	
<i>Agropyron intermedium</i> Beauv.	$\frac{2}{45}$ ; <sup>12</sup>	Minute uredinia; flecks indistinct	$\frac{0}{25}$	

<sup>1</sup> Hoerner, G. R. Biologic forms of *Puccinia coronata* on oats. *Phytopath.* 9: 309-314. *Pl.* 19-20. 1919.

<i>Agropyron repens</i> (L.) Beauv.	$\frac{0}{49}$		$\frac{0}{98}$	
<i>Agropyron smithii</i> Rydb.	$\frac{0}{47};^{10}$	Flecks distinct; extensive dead areas	$\frac{0}{54}$	
<i>Agropyron tenerum</i> Vasey	$\frac{0}{23}$		$\frac{0}{35}$	
<i>Agrostis alba</i> L.	$\frac{0}{559}$	*	$\frac{37}{510}$	Light to moderate
<i>Agrostis stolonifera</i> Vasey	$\frac{0}{65}$		$\frac{26}{273}$	Light to moderate; strong flecks
<i>Alopecurus geniculatus</i> L.	$\frac{164}{620};^8$	Moderate uredinia; flecks distinct *	$\frac{57}{77}$	Moderate
<i>Alopecurus pratensis</i> L.	$\frac{3}{191};^{23}$	Minute uredinia; distinct whitened areas*	$\frac{154}{200}$	Moderate to heavy
<i>Anthoxanthum odoratum</i> L.	$\frac{8}{102};^{68}$	Moderate uredinia; flecks distinct	$\frac{76}{183}$	Moderate
<i>Arrhenatherum elatius</i> (L.) Beauv.	$\frac{1}{121};^3$	Minute uredinia,* flecks indistinct	$\frac{19}{211}$	Light to moderate
<i>Avena fatua</i> L.	$\frac{48}{60}$	Heavy*	$\frac{83}{84}$	Heavy
<i>Bromus erectus</i> Huds.	$\frac{1}{64};^4$	Minute uredinia; flecks distinct*	$\frac{3}{35}$	Light
<i>Bromus inermis</i> Leyss.	$\frac{1}{77};^5$	do.	$\frac{0}{40}$	
<i>Bromus purgans</i> L.	$\frac{0}{23}$	*	$\frac{1}{23}$	do.
<i>Bromus tectorum</i> L.	$\frac{1}{164};^{13}$	Minute uredinia	$\frac{194}{194}$	Light to moderate
<i>Calamagrostis canadensis</i> (Michx.) Beauv	$\frac{0}{3};^2$	Flecks indistinct	$\frac{34}{65}$	Moderate

<i>Cynosurus cristatus</i> L.	$\frac{0}{43}, 2$	do.	$\frac{0}{90}$	
<i>Dactylis glomerata</i> L.	$\frac{16}{32}, 1$	Moderately heavy	$\frac{70}{98}$	Heavy
<i>Danthonia spicata</i> (L.) Beauv.	$\frac{0}{45}$		$\frac{0}{36}$	
<i>Elymus canadensis</i> L.	$\frac{0}{80}, 29$	Flecks distinct	$\frac{16}{285}$	Small uredinia; weak
<i>Elymus robustus</i> Scribn. and J. G. Sm.	$\frac{2}{59}, 17$	Moderate uredinia; telia, flecks distinct	$\frac{4}{158}$	Small uredinia
<i>Elymus virginicus</i> L.	$\frac{0}{15}$		$\frac{0}{160}$	
<i>Festuca elatior</i> L.	$\frac{0}{87}$		$\frac{12}{184}$	Weak
<i>Festuca ovina</i> L.	$\frac{2}{129}, 6$	Minute uredinia; flecks indistinct	$\frac{0}{54}$	Moderate
<i>Festuca rubra</i> L.	$\frac{1}{150}, 38$	Minute uredinia; flecks indistinct	$\frac{0}{75}$	
<i>Holcus lanatus</i> L.	$\frac{3}{135}, 12$	do.	$\frac{81}{233}$	Moderate; small uredinia
<i>Hordeum jubatum</i> L.	$\frac{1}{38}, 9$	Minute uredinia; flecks distinct	$\frac{0}{55}$	
<i>Hordeum pusillum</i> Nutt.	$\frac{15}{94}, 47$	Moderate uredinia; flecks distinct	$\frac{4}{28}$	Small uredinia
<i>Hordeum vulgare</i> L.	$\frac{3}{73}, 25$	*Very minute uredinia; flecks distinct	$\frac{69}{678}$	Moderate to heavy; minute uredinia
<i>Lolium italicum</i> R. Br.	$\frac{0}{109}, 2$	Flecks indistinct*	$\frac{2}{173}$	Weak
<i>Lolium perenne</i> L.	$\frac{0}{70}$		$\frac{5}{191}$	do.

<i>Lolium temulentum</i> L.	$\frac{9}{127}$ ; 54	Minute uredinia; dead colorless areas extensive; very hypersensitive	$\frac{16}{55}$	Moderate
<i>Phalaris canariensis</i> L.	$\frac{0}{37}$		$\frac{17}{19}$	do.
<i>Phleum pratense</i> L.	$\frac{30}{80}$ ; 34	Moderately heavy; flecks distinct	$\frac{16}{186}$	Weak
<i>Savastana odorata</i> (L.) Scribn.	$\frac{0}{10}$		$\frac{0}{21}$	
<i>Secale cereale</i> L.	$\frac{0}{111}$ ; 29	Flecks distinct	$\frac{21}{413}$ ; 3	Minute uredinia
<i>Triticum vulgare</i> Vill.	$\frac{0}{69}$ ; 12	do.	$\frac{0}{457}$	

In some of the trials urediniospores were applied in water suspension by means of an atomizer.

*Summary:* (1) There seem to be slight differences in infection capabilities between the four specimens of *P. coronata* Cda. used in these experiments, though from the evidence at hand, no conclusions have been drawn (2) There are a number of common hosts for *P. coronata* Cda. and *P. graminis avenae* Erikss. and Henn. (3) The crown rust of oats in the United States has a host range under greenhouse conditions particularly that is much more extensive than formerly has been thought to be the case.

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## BRIEFER ARTICLES

### SOME RECENT INVESTIGATIONS ON THE CONTROL OF *SCLEROTINIA* *LIBERTIANA* IN THE GREENHOUSE ON THE MUCK FARMS OF BERGEN COUNTY, NEW JERSEY

R. F. POOLE.<sup>1</sup>

WITH THREE FIGURES IN THE TEXT

*Sclerotinia libertiana* (Fuckel) has for several years been the cause of a very destructive "damping off" of young celery plants in the greenhouses on the muck farms of Bergen County, New Jersey. Although the infection has been greatest on muck and highly organic soils used for seed beds, losses have also been very general on other types of greenhouse soil and sash beds in the state. Celery plants are grown in these greenhouses from February to June, through a period in which temperature and moisture conditions continually fluctuate and are very often favorable for the rapid development of the fungus. All varieties are susceptible. In some cases a large percentage of the plants are "damped off," in a few days. Though the disease is very destructive to celery under greenhouse conditions, the field losses throughout this state have been small.

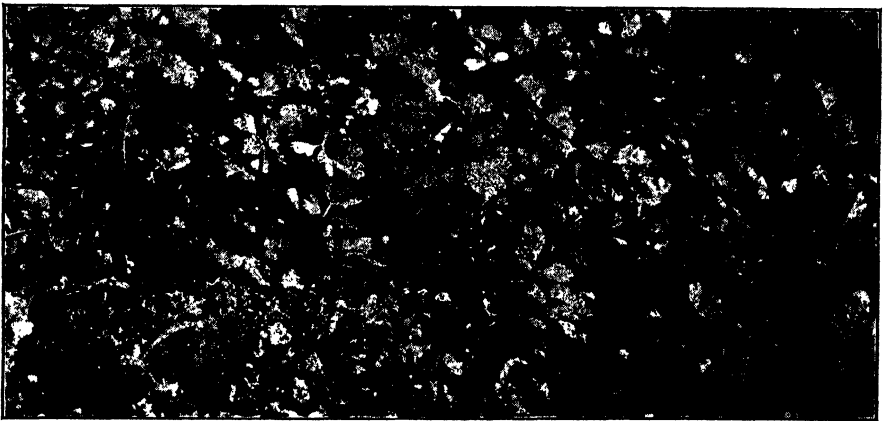


Fig. 1. Celery seedlings being killed by *Sclerotiana libertiana*. Entire beds are sometimes killed in a few days.

<sup>1</sup> Paper No. 52 of the Journal Series, New Jersey Agricultural Experiment Station, Department of Plant Pathology.

*Sclerotinia libertiana* has caused "damping off" of a large number of vegetable and truck crops, where the seedlings are grown in hot beds. Peppers, spinach, rhubarb, lettuce, carrots, beans and a score of other plants are attacked by this organism.



Fig. 2. Decay of mature celery plants, in boards for blanching, caused by *Sclerotinia libertiana*.

#### DESCRIPTION OF THE DISEASE

Celery is attacked during all stages of growth in the greenhouse beds, but infection is most destructive about the time that the plants are large enough to set in the field. The attack is usually centered at first on the stalks just above the ground. Infected stalks decay quickly with a wet soft rot. Later the tops fall over and the fungus spreads to this part of the plant and to adjacent plants (Fig. 1). The fungus

growth appears as a white cottony mass usually abundant and easily seen on decaying plants. Infection is first of all detected in isolated areas of the beds; the apothecia develop abundantly here and spores are discharged in great numbers. The plants become infected subsequently and decay in circular areas, finally spreading throughout the house. Infection usually occurs in a few days following the presence of the apothecia in the beds. The "watery soft rot" or "foot rot" condition is sometimes seen on large celery plants in the field (Fig. 2). The infected portion appears pinkish at first, later rotting away and turning into a dark gray, soft mass. Decay and destruction of the plants are largely determined by environmental conditions.

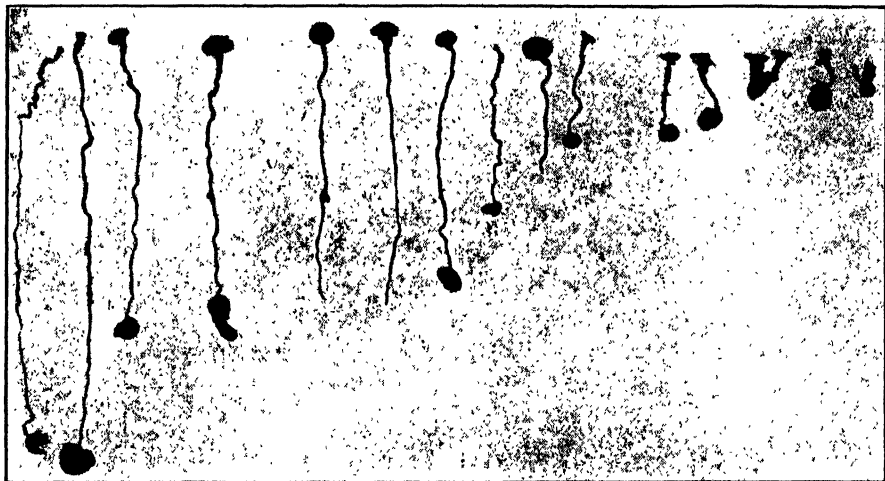


Fig 3. Apothecia of *Sclerotinia libertiana* produced by sclerotia at various depths in the soil;  $\times$  about  $\frac{1}{4}$ .

#### THE LIFE HISTORY OF *SCLEROTINIA LIBERTIANA* IN THE MUCK SOIL OF GREENHOUSE BEDS

It is not difficult to trace the life cycle of *S. libertiana* in the muck soil of the greenhouse beds. The soil is prepared for sowing seed about the first of February. From June to February the soils are allowed to air dry to a dusty powder in the greenhouse beds. During this period little if any fungus growth takes place, but soon after when the moisture and temperature conditions are made favorable for the growth of the plants (February to June) the sclerotia begin to develop apothecia, which rise to the surface from various depths. Some of the stipes were found to be three inches in length (Fig. 3) and it seems very possible

that apothecia can emerge from even a greater depth through the loose muck soil.

Infection occurs almost simultaneously with the formation of apothecia and the development and liberation of ascospores. After the plants are attacked, the mycelium spreads rapidly. The size and quantity of sclerotia formed is dependent on the size and density of the plants, likewise on temperature and moisture conditions. High humidity and temperature favor the development of the fungus. As a general rule the plants are small when they become infected, and the period of fungus growth is short. Therefore, a very large number of small sclerotia are produced. Large sclerotia may occur along with these. In houses that have become infected there is usually one severe outbreak of the disease each year. The sclerotia are produced after the mycelium has developed. The mycelium appears at first as a white cottony mass which is very prominent over the surface of the greenhouse beds. Under such a condition the sclerotia become fairly well distributed. About the time the sclerotia mature, celery plants can be grown in the open and are reset in the field. The houses are then closed from June to February during which term the soil remains dry and the sclerotia dormant until moisture is again applied in February when the sclerotia produce apothecia and the life cycle of the fungus recommences. It is then definitely known when the fungus develops in the greenhouse.

The fungus is carried into the greenhouse beds in soil from fields where lettuce is grown or adjacent fields. Lettuce is more susceptible to attack in the field than celery. Dissemination of *S. libertiana* is due very largely to the growing of lettuce, on the cultivated muck soils in rotation with celery.

#### CONTROL METHODS

Experiments over several years have shown that the disease can be controlled in the greenhouses by using virgin soils in the beds. Infected soils were thrown out, and replaced with soil selected from fields where lettuce had not been previously grown. At times even under the most sanitary conditions, the fungus is carried into the houses in infected soils. To make certain of control conditions, the soil was sterilized the first year with formaldehyde. After this the treated soil can be used for several years without change. Other diseases and cultural conditions sometimes make it necessary to remove and replace the soil in the greenhouse beds annually.

Formaldehyde properly applied to infected soils in the greenhouse beds has been very effective in controlling the disease. The best results were obtained as follows: by watering down the beds so that all particles

were wet and then applying formaldehyde, 3 pints to 50 gallons of water, to the soil at the rate of one gallon to the square foot. The soil was treated about two weeks before the seed were sown. When burlap bags were placed over the treated soils for 24 hours, the treatment was more effective than when no bags were used to retain the formaldehyde fumes. The seed germinated and the plants grew much better on treated soils which were pulverized and aired well several days before the seeds were sown. Tests in which less water was used failed to control the disease. The essential thing to remember in applying formaldehyde is to mix the commercial product in enough water to insure thorough distribution so that the formaldehyde will come in contact with all parts of the soil.

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#### THE CONTROL OF ANGULAR LEAF SPOT OF COTTON

C. A. LUDWIG

A method for the control of angular leaf spot of cotton was developed a few years ago at this station as a result of the work of Rolfs (3) and Faulwetter (2). At the time circumstances prevented more than a single year's field trial of the treatment. During the present season (1921), however, the test has been repeated; and the object of this paper is to set forth the results secured.

It will be recalled that the treatment consists in stirring the seed in strong sulphuric acid until the lint is removed, washing, sterilizing for about ten minutes in a one to one thousand solution of mercuric chloride, washing again, and drying.

In our trials this year a sufficient quantity of seed was divided into two lots, one of which was treated and the other left untreated. The seed used was of the Cleveland Big Boll variety. It was grown on the experiment station farm in 1920 and came from plants abundantly affected with angular leaf spot. The tests were conducted at the experiment station at Clemson College and at the Pee Dee branch station at Florence, South Carolina. At each station the trials were carried out in triplicate by a plan only slightly modified from that previously described by Faulwetter (2). At Florence there were six 20 x 181.5 ft. plots of cotton alternating with five 24 x 181.5 ft. plots of corn and velvet beans. The total area used was one acre, which allowed one half acre for cotton and one half acre for the inter-plots. At Clemson College the plots were approximately 35 ft. long for the cotton and the same

for interplots of corn. The width varied with that of the terrace used, from 28 ft. at one end of the area to 40 ft. at the other. Counts were made at intervals to determine the number of affected plants in each plot, any plant with one or more spots being counted as affected. The following table gives the results secured, the results on corresponding plots being combined:

TABLE 1. *Control of angular leaf spot of cotton by treating seed with sulphuric acid and mercuric chloride.*

At Clemson College, S. C. Date of planting, May 5, 1921.

DATE	TREATMENT	AFFECTED PLANTS	CLEAN PLANTS	% AFFECTED PLANTS
June 8	Treated	None	632	0.0
June 8	Check	224	406	35.6
June 21	Treated	None	453	0.0
June 21	Check	294	168	63.6
July 22	Treated	6	193	3.0
July 22	Check	116	105	52.5

At Florence, S. C. Date of planting, April 27, 1921.\*

DATE	TREATMENT	AFFECTED PLANTS	CLEAN PLANTS	% AFFECTED PLANTS
June 2	Treated	2 (?)	593	.3 (?)
June 2	Check	125	391	24.2
June 15	Treated	None	551	0.0
June 15	Check	311	356	46.6
July 9	Treated	7	143	4.7
July 9	Check	101	49	67.3
July 28	Treated	11	139	7.3
July 28	Check	128	22	85.3

\* The stand secured was poor and the vacant places were later replanted.

It can be seen from the foregoing data that angular leaf spot was kept out of the plots planted with treated seed until the parasite had had time to enter from outside the plots. The two doubtful infections recorded at Florence on June 2, are really to be considered as having some other cause than *Bacterium malvacearum*, because they were actually very doubtful.

On Sept. 1st some further notes were taken on the prevalence of angular leaf spot at Clemson College. No counts were made, but there still remained enough difference in infection to show a beneficial result from the treatment. No plot was very seriously infested, however, owing probably to the unusually dry weather of the growing season.

Some other tests of the treatment have recently been reported by Elliott (1), of the Arkansas experiment station. In these cases, also, the cotton raised from treated seed remained free from angular leaf spot until the parasite had entered from outside sources. In one case in

particular, the place of entry was along a footpath traversing both the noninfested field and an infested one, thus indicating that laborers may be one means of spreading the organism.

These results all seem to confirm the reports previously issued by this station and indicate that treatment of cotton seed with sulphuric acid and mercuric chloride is an absolute control for angular leaf spot of cotton.

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CLEMSON COLLEGE, S. C.

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## PHYTOPATHOLOGICAL NOTES

*The Biologische Reichsanstalt at Berlin-Dahlem and the plant protection service in Germany.* Last year the Biologische Reichsanstalt has been enlarged and somewhat changed under the supervision of its new director Prof. Dr. Appel, well-known also in the United States America by his stay there. Some practical laboratories have newly been erected as an economic section in order to serve more the now so important requirements of disease control. The laboratories for botany, physiological zoology, bacteriology and chemistry till now remain as a scientific section.

The new economic section I comprises the laboratories IA for general plant-protection with the inquiry office, IB for cereals and forage crops, IC potatoes, ID sugar beets, IE vegetables, market and garden plants, IF orchards and viticulture, IG forestry, IH pests of stored products, IJ practical soil bacteriology, IK practical agricultural chemistry, IL phenology and meteorology, IM bee diseases and IN control of insecticides and fungicides and the collections.

Beyond that, the library has been placed under scientific control, so that the librarian is at the same time the editor of all publications of the Institute. Since July a new monthly periodical is issued under the title of "Nachrichtenblatt für den deutschen Pflanzenschutzdienst." The Institute has not only erected the practical laboratories but also in the last few years diverse dependencies which have been transferred to important districts for the research of diseases and pests of certain cultivated plants. The most extensive of them in Naumburg a. S. treats the pests of fruits, vine and oliverous plants, especially with the research of the phylloxera, another dependency at Aschersleben is for vegetables and garden plants, others again in Trier, Stade, and Zittau serve the research of diseases and pests of vine, fruit-trees and sylviculture.

Of late in Germany there has been frequent discussion of the question of the relations between botany and entomology to the disease control which are there, the same as in many other countries, comprehended by the name of phytopathology. Therefore it must be stated that the various divisions of natural science have remained strictly separated in the scientific section, which serves the scientific research. Whereas they are united in the laboratories of the economic section, which is classified after the cultivated plants and serves the practical disease control. In these laboratories zoologist, botanist and bacteriologist



work together according to requirement and it is desirable that every one of them should master as far as possible the entire domain of phytopathology. The Forschungsinstitut for the cultivation of potatoes which was up till now under the supervision of Prof. Dr. Appel has in the present year been joined to the Institute. It comprises 4 sections, physiology, pathology, anatomy-microchemistry and agriculture.

In general the organization of the disease control has remained unaltered in the separate countries and provinces. At present there are existing 30 principal sections for disease control which are at the same time research institutes for the survey of the plant diseases; they are united with phytopathological or agricultural experiment stations. Every principal section has numerous districts and to these collectors are joined in the several places for the observation and information service. Numerous experts have specially been educated who devote themselves in the separate places to the restraint of diseases or give instructions to the people. In this way the practical disease control has lately been greatly enlarged in many parts of Germany. It is remarkable that this progress has especially been made in those countries which have many small holdings.—H. MORSTATT, BERLIN-DAHLEM.

*A new source of supply of pure cultures of fungi.*—In the Gardener's Chronicle of September 17, 1921, p. 133-4, is a notice of special import to mycologists and plant pathologists regarding this subject. In view of the successful arrangements made for maintaining a national (British) collection of type cultures of bacteria and protozoa at the Lister Institute, the British Mycological Society proposes utilizing the same machinery to maintain a similar national collection of cultures of fungi, chiefly those of economic importance or educational significance. The following quotation explains the plan:

"Cultures will be supplied on demand, so far as possible, to workers at home and abroad, and, as a rule, a small charge will be made to defray the cost of media and postage. Annual lists of the fungi in the collection will be published in the Transactions of the British Mycological Society. A set of type slides of fungi will be kept in the Botanical Department of the Museum in addition to a working set at the Lister Institute. It is requested that all communications be addressed to: The Curator, National Collection of Type Cultures, Lister Institute, Chelsea Gardens, London S. W. I."

This British center for the maintenance of cultures of fungi, together with the Centraalbureau voor Schimmelcultures, Baarn, (formerly at Amsterdam) Holland, serves a real need of investigators. While these organizations place their resources at the command of all mycologists, they are of chief convenience to Europeans, and their distance prohibits most effective service to workers in America. It is more imperative

than ever that a cooperating organization of similar character should be established in this country where the need for such cultures has been most keenly felt for a long time. If this cannot be done through government agencies some scientific society or other organization should undertake it.—C. L. SHEAR.

*The corn ear worm and kernel rot of corn.*—One of the most injurious pests on field corn this season has been the corn ear worm with its destructive effects on the ear, which is causing difficulty for many growers in selecting perfect ears for show purposes. The injury upon the ear produced by the worm has been consistently followed with infection by *Fusarium moniliforme* and *Cephalosporium sacchari*. With the corn in the milk stage, entire kernels were usually found eaten but as the corn ripened it was most common to find only the top of the kernels eaten. The first point of attack by the worm upon the ear is usually the tip and it gradually feeds downward, often eating two or three rows of kernels, sometimes to half the length of the ear. In many fields the number of ears attacked will average thirty to fifty per cent and it was not uncommon to find fields showing eighty to ninety per cent infestation of ears. It has been estimated in some instances that twelve per cent of the weight of grain of infested ears is destroyed.

Garman and Jewett (Kentucky Bul. 187, 1914) state, "for while some molds, such as *Diplodia zeae*, invade corn ears without help, the majority are incapable of doing so, and most of those to be observed on the so-called moldy corn are present only because the corn worm admits them along the path it makes in invading the ears." It is of peculiar significance that *F. moniliforme* and *C. sacchari* have been found so commonly following injury of the corn ear worm in view of the prevalence of the fungi carried internal of seed as reported by the writers. Where infection has become established with injured kernels they were completely rotted and light brown to purplish in appearance. Adjacent kernels apparently uninjured were found commonly rotted and in some instances there were as many destroyed because of infection as originally injured by the worm. The fungus produced an excessive superficial growth and with an abundant sporulation there was present a favorable source of inoculum. Kernels only partly infected and adjacent to those injured by the worm showed a conspicuous symptom in the reddish pink discoloration of the crown. In instances where infection of *F. moniliforme* occurred these pink symptoms were of a radiating streak-like character extending from the crown for one-eighth to one-quarter inch down the length of the kernel. This symptom of infection by *F. moniliforme* was always associated with infestation of the corn ear worm.

Free hand sections and poured agar plates of such symptoms proved the presence of *F. moniliforme*. With mature kernels not attacked by the worm the infection and discoloration was confined principally to the pericarp as very little evidence was found of infection progressing into the endosperm. The manner of entrance of the fungus in uninjured kernels was not determined but would appear to be correlated with a lessened resistance in the crown tissue of the kernel or through the point of style attachment. Mr. Claude Phillips, one of our graduate students, made some poured agar plates from washings of worms removed from infested ears. Such plates showed abundant colonies of *F. moniliforme*. Similar work was carried out with the same worms disinfected and crushed but the results were negative. This indicates the worm is capable in its progress of injury on the ear of furthering the source of infection when established.

It was also common to find kernels associated with worm injury which showed a solid pink color involving usually the upper half of the kernel. This symptom was easily distinguished from the infection by *F. moniliforme* and the symptoms often involved the entire endosperm as observed in free hand sections of such kernels. Cultures of these kernels showed a consistent prevalence of a bacterial organism. We have not determined whether this symptom is the result of secondary infection of the bacterial organism or an enzymatic action as the result of injury by the worm. At least the correlation of this symptom with the infestation indicates the corn ear worm is indirectly responsible for its presence.

The injury to the ear by the worm is not alone the important effect upon the corn plant. Seedling plants eight inches high are attacked at the growing point and in some instances replanting has been necessary because of the resultant death to seedlings. Plants just before emergence of tassel also show evidence of the worm injury by the extensive eating of the young rolled leaves. This injury to young and half grown plants would seem to offer favorable openings for infection of the different fungi associated with the corn rot diseases but in no instance has there been found any symptoms of infection during extensive field observations.—J. F. ADAMS AND T. F. MANNS.

**ABSTRACTS OF PAPERS PRESENTED AT THE THIRTEENTH  
ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL  
SOCIETY, TORONTO, CANADA, DECEMBER 28 TO 31,  
1921.**

*Ophiobolus cariceti* (Berk. & Br.) Sacc., cause of take-all of wheat. H. M. FITZPATRICK,  
H. E. THOMAS, AND R. S. KIRBY.

The discovery in Monroe County, New York, of perithecia of a species of *Ophiobolus* on wheat plants showing characteristic symptoms of the take-all disease was reported in Science in October, 1920. Subsequently additional collections of the fungus were made in scattered localities. It has been grown in pure culture and inoculations have demonstrated it to be the causal organism of the take-all disease. It has been compared with various foreign collections of material, and found to be identical with the species known as *O. graminis* in England, France, Japan, Italy, and Australia. Study of type and other authentic material of *O. cariceti* (Berk. & Br.) Sacc. from Kew shows this species to be identical with the take-all organism. Since this name antedates *O. graminis* Sacc. it is accepted for the species and *O. graminis* is regarded as a synonym. Berlese's assertion that *O. cariceti* is identical with *O. eucryptus* has been found to be incorrect. A paper embodying the results of the investigation and containing a detailed description and illustrations of the organism will appear in the next number of Mycologia.

*The take-all disease of cereals and grasses.* R. S. KIRBY.

*Ophiobolus cariceti* has been demonstrated to be the cause of the take-all disease previously reported as occurring in New York on wheat, rye, and *Agropyron repens*. The causal organism was isolated, grown in pure culture on numerous media, and typical perithecia were produced. The fungus is confined to the roots and the lower internodes of the host. Perithecia are produced in abundance, over one hundred having been found on single culms. The fungus is not disseminated in the seed, but lives for varying lengths of time in the soil and on the straw. The disease is more destructive on alkaline than on acid soils. Correlated with this the growth of the organism has been shown to be better on alkaline than on acid media. On corn meal agar growth begins at about 4.5 pH and increases gradually to 8.1 pH, the point at which maximum growth occurs. As the results of inoculations in the greenhouse typical perithecia were produced on wheat, barley, and rye, and on one or more species of the following genera of wild grasses: *Agropyron*, *Bromus*, *Elymus*, *Festuca*, *Hordeum*, *Hystrix*, *Lolium*, and *Phalaris*.

*Foot-rot disease of wheat in Kansas.* H. H. MCKINNEY AND L. E. MELCHERS.

This disease was first noticed in Kansas in 1920. In 1921 a survey showed the disease to be present in seventeen fields on eleven farms located in Dickinson, Saline, Riley, and

Cheyenne Counties. Both hard and soft wheats were affected. In some fields the disease caused almost a total crop loss.

The disease occurred in scattered, circular, or irregular spots, of various sizes. These occurred without regard to topography or soil conditions. The first indication of the disease was a yellowing of affected plants shortly after resumption of growth in the spring. This yellowing continued until plants approached maturity. Diseased plants became bleached and remained stiff and upright. A distinct black scale or plate of interwoven mycelium developed at the base of many of the diseased plants, between or underneath the leaf sheaths next to the culm. The disease resembles the true take-all disease occurring in this country and described in foreign literature, but differs in several respects from the so-called take-all disease occurring in Illinois and Indiana. The cause of the disease in Kansas remains unknown. (U. S. Bureau of Plant Industry and Kansas Agricultural Experiment Station cooperating.)

*The Helminthosporium disease of wheat and the influence of soil temperature on seedling infection.* H. H. MCKINNEY.

The *Helminthosporium* disease of wheat is now known to occur in the spring and winter wheat belts and in the western intermountain region. Under certain conditions the disease causes considerable crop loss. So far, practically all the strains of *Helminthosporium* isolated from wheat appear to be very similar if not identical. Comparisons with *Helminthosporium sativum* P. K. and B. isolated from spot blotch lesions on barley, show no marked morphological differences. Cross inoculations made with the wheat and barley strains of the organism on the foliage and the underground portions of the tillers of wheat and barley show that both strains produce the typical symptoms on both hosts. Field observations indicate that the disease is influenced by environmental conditions. Controlled soil-temperature experiments, conducted in the "Wisconsin temperature tanks," and field experiments show that seedling infection in both spring and winter wheat and in spring barley is greatest at relatively high temperatures. The optimum temperature apparently lies between 26° and 28° C. This is very near the optimum for the rate of growth of *H. sativum* in pure culture. (Office of Cereal Investigations, U. S. Department of Agriculture, and the Wisconsin Agricultural Experiment Station cooperating.)

*A seedling blight caused by Fusarium culmorum var. lelei Sher.* JESSIE P. ROSE.

In conducting germination tests with treated and untreated wheat of 18 varieties during the seasons of 1918-19 and 1920 at Corvallis, Oregon, the writer observed a high seedling mortality with which different fungi, particularly *Fusarium culmorum* var. *lelei*, were found associated. *Fusarium culmorum* var. *lelei* was found to penetrate the endosperm and young seedlings, and produced a typical seedling blight. Inoculation experiments with this fungus conducted in laboratory, greenhouse, and field, using both treated and untreated spring and winter wheats, showed from 3 to 98 per cent of seedling blight, depending upon the condition of the seed and the environmental factors. The severity of the blighting depended upon soil moisture and temperature conditions, upon the amount of injury received from threshing, and seed treatment injury as correlating factors. *Fusarium culmorum* var. *lelei* was isolated consistently from diseased seedlings of wheat, oats, barley, and rye grass obtained from different parts of Oregon. (Cooperative investigations between the Oregon Agricultural Experiment Station and the U. S. Office of Cereal Investigations.)

*Diplodia zeae* as an ear and root parasite of corn. EDWARD E. CLAYTON.

Work in Ohio during the winter of 1920 and 1921 showed that the fungus *Diplodia zeae* was very prevalent in seed corn. Many ears that appeared healthy were found to be infected with this organism, and often these infected ears were partly dead. Seed from such infected ears was planted in crocks and the soil held at the temperatures of 15 to 18°, 21 to 24°, and 29 to 31° C. With the soil temperatures from 21 to 24° C., the roots of the young plants were severely rotted; with the soil temperature 29 to 31° C., the fungus was only slightly less active; but at 15 to 18° C., the plants were not affected by the root rot. These temperature tests were repeated in the fall of 1921, using corn inoculated with *Diplodia* in pure cultures. Similar results were secured in ear to row field tests, where seed from healthy ears was compared with seed infested with *Diplodia*. A poor stand of plants and a poor yield of corn resulted from the *Diplodia* infection. During the summer of 1921, ears of corn in the field were inoculated with *Diplodia*, the fungus having been isolated from rotting roots. The inoculations made August 31 resulted in complete rotting of the ears. Practically all of the kernels in these ears were dead. Inoculations made September 20 gave a high percentage of infested ears, which, however, in most cases showed no symptoms of ear rot. Very few of the kernels in these ears were dead. Regarding the occurrence of natural infestation, the following data has been secured:

*Time of natural infection of ears in field, 1921.*

Date Corn picked	Percentage of ears infected with <i>Diplodia</i>
August 31.....	5%
Sept. 20.....	9%
Oct. 10.....	18%
Oct. 31.....	19%

*Diplodia* of corn in Iowa. L. W. DURRELL.

Studies on *Diplodia zeae* in pure culture indicate that a high humidity is necessary for optimum growth. Further temperature relations as determined on pure cultures under controlled conditions indicate a very high optimum temperature for growth. The cardinal temperatures are, optimum 29 to 31° C., maximum 34 to 36° C., and minimum 10 to 12° C. No growth occurs above 36° C., nor below 10° C. The crest of the temperature curve varies little between 27 and 31° C., with the peak nearer 30° C. The cultural reactions of the fungus correlate well with the combination of high temperature and humidity prevailing in central Iowa the past season. The infection of corn by *Diplodia* is largely through the nodes. Spores drop between sheath and stalk and later attack the corn tissue. Not all nodes are thus affected. Often one node will be found infected, the next two clean, the third above infected, and so forth. No evidence has been found that the fungus migrates up the stalk, but many plantings tend to show that the infection entering the node often works both ways from that point. Shanks of the ear are frequently found heavily infected with *Diplodia*. Cultural studies indicate that these diseased shanks became infected from the node. The fungus sometimes migrates from the shank to the butt of the ear. In the large majority of cases this year infection began at the butt of the ear. Many ears during the period of high humidity actually stood in water, the flask-like sheaths retaining water sufficient in some cases to sprout the grain. Under such conditions if *Diplodia* were present the entire ear and husk became completely infected. No constant relation has been found between *Diplodia* infection and the broken shanks and stalks.

*The relation of soil temperature to the development of the seedling blight of corn caused by Helminthosporium sp.* W. G. STOVER.

A species of *Helminthosporium*, isolated from living corn plants, was found to cause a marked seedling blight of corn. All inoculations were made by immersing corn seeds in a spore suspension before planting. Mesocotyl, cotyledonary node, and seminal roots were rotted. The diseased region was dark brown to deep black and usually shrunken. The optimum temperature range for the growth of the fungus on potato dextrose agar was 21 to 29° C., with the maximum growth at approximately 26° C. Inoculated and uninoculated corn was grown in the temperature tanks at the University of Wisconsin at temperatures of 8 to 36° C., with 4° intervals. The optimum soil temperature for growth of corn roots was apparently 20 to 24° C., and for growth of tops, 24 to 28° C. Seedling blight developed to some extent at all temperatures tried, but was much more marked from 16 to 24°, and especially at 20° C., where 100 per cent of the plants in several trials were attacked, and the ratio of severely diseased to slightly diseased seedlings was 9 to 1. Results of one trial indicated that a relatively high moisture content of the soil is favorable for the development of the disease.

*Treatment of seed to control root and stalk rots.* B. B. BRANSTETTER.

An experiment carried on at the Missouri Agricultural Experiment Station with seed corn infected with root and stalk rot organisms gave results indicating that seed treatment is effective in reducing the amount of root and stalk rot in the field. Corn gathered from various sources was germinated in a table germinator and classified as heavily infected, moderately infected, and slightly infected seed according to whether the seed showed a high, moderate, or slight per cent respectively of seedlings affected with root rots. Six rows 400 feet long with hills 3 feet apart were planted to each of these three groups of seed, but the seed in three rows of each group were first disinfected by immersing the seed momentarily in alcohol and then in mercuric chloride solution, 1: 1000, for one hour. In September while the healthy stalks were still green, these plants were inspected for root and stalk rots. Rows planted from heavily, moderately, and lightly infected seed averaged respectively 27.4, 16.4 per cent of diseased plants while seed from the same lots disinfected averaged respectively 15.5, 12.5, and 8.9 per cent of diseased plants. This indicates first, that the relative amount of disease in the field is roughly proportional to the root rot shown on the table germinator; and second, that disinfection of the seed as above described materially reduces the amount of root and stalk rot in the field.

*The improved rag-doll germinator box.* R. S. KIRBY.

A germinator box was devised which would make possible the separation with certainty of healthy seed corn from *Fusarium*-infected ears. The germinator consisted of a large box with double walls. The air inside this box was kept at complete humidity and constant temperature by a tank of water heated by electric elements controlled by a thermostat. The tank contained seven inches of water and rested on the bottom of the box. A wire screen was supported seven inches above the surface of the water. The space above the screen was divided into compartments, which were again subdivided by wires into spaces in each of which a rag doll would stand upright. Three inches above the dolls was a cover for each compartment. These in turn were covered with the lid of the box. The box was 70 x 38 x 38 inches and had a capacity of 6000 ears. Running the germinator at a temperature of 85° F., 12,500 ears of corn were tested in the spring of 1921 and graded according to the presence of *Fusarium*. By the use of

this box it was possible to obtain a grade of corn which had no *Fusarium*-infected kernels, as shown by a subsequent test on media.

*The effect of fertilizers on the development of stem rust of wheat.* E. C. STAKMAN AND OLAF S. AAMODT.

Since 1913 experiments have been made to determine the effect of artificial and natural fertilizers on amount of rust developed on susceptible and resistant varieties of wheat when grown on several soil types in different parts of Minnesota, under heavy artificially-induced epidemics, and under natural field conditions. The experiments were supplemented by controlled greenhouse experiments. The amount of rust was not changed directly by any fertilizer or combination of fertilizers, although date of maturity, degree of lodging, crinkling, shrivelling of seed, percentage of yellow-berry, and yield were affected profoundly. Leaf rust developed most abundantly on plats fertilized with nitrogen. On properly fertilized soil wheat yielded well regardless of heavy stem-rust attacks. In one plat fertilized with 250 pounds acid phosphate and 500 pounds potassium sulfate, Haynes Bluestem (Minn. 169) yielded 31 bushels per acre even though the rust infection was 88 per cent. Plants in plats fertilized with 1000 pounds sodium nitrate had 80 per cent of rust, but the acre yield was only 8 bushels. Neither potassium nor acid phosphate counteracted the effect of nitrogen in lowering yield on some types of soil. The effect of fertilizers on general character of plant growth and yield varied on different soil types, but the soil type, with or without fertilization, did not directly influence the amount of stem rust. (Cooperative investigations by the Minnesota Agricultural Experiment Station and the Office of Cereal Investigations, U. S. Department of Agriculture.)

*The effect of rust infection upon the water-requirement of wheat.* FREEMAN WEISS.

Wheat was grown to maturity in quartz sand cultures which were supplied with various combinations of mineral nutrients added in solution. An artificial epiphytotic of leaf rust, *Puccinia triticina*, was induced in one series of stem rust, *P. graminis tritici* in a second; while a third was maintained free from infection. Rust infection of either type resulted in lowered water economy of the plant, whether the dry matter of tops or heads is considered. The actual quantity of water transpired possessed significance in relation to infection only when the correlative production of dry matter was taken into account.

The addition of NaCl or  $\text{NaH}_2\text{PO}_4$  to the basic 3-salt nutrient solution failed to modify the susceptibility of the host.  $\text{NaNO}_3$  resulted in readier infection, but did not predispose the host to greater injury. KCl retarded infection, but when used in unbalanced proportions also markedly diminished yield of grain.  $\text{CaCl}_2$  and  $\text{MgCl}_2$  resulted in less ready and less severe infection. The former brought about greater water economy—about 10 per cent for tops and 40 per cent for grain. The Shive solution R3C3 as here used did not suffice for best development of wheat to maturity. The addition of .0085 and .0171 gram-molecules of  $\text{NaNO}_3$  per liter of solution resulted in a 10 per cent increase in weight of tops, and .0171 gram-molecules of  $\text{CaCl}_2$  increased both tops and grain, the latter by 60 per cent.

*Inheritance of resistance to black stem rust in crosses between varieties of common wheat (*Triticum vulgare*).* LEO E. MELCHERS AND JOHN H. PARKER.

Crosses were made in 1917, using three rust-resistant varieties of winter wheat, Kanred, P1066, and P1068; and three susceptible spring wheat varieties, Marquis,



Preston and Haynes Bluestem. The  $F_1$  plants proved to be resistant when inoculated with a strain of *Puccinia graminis tritici*, to which Kanred is resistant. All inoculations were made in the greenhouse at time of heading. In the  $F_2$  generation, 1921 plants were grown in the greenhouse and each plant was inoculated with stem rust. Definite segregation occurred. There were 1,375 plants which were classified as resistant and 546 described as susceptible. Both resistant and susceptible  $F_2$  plants were tested. An  $F_3$  generation of approximately 1,750 plants of the Kanred x Marquis cross was grown. The twenty  $F_2$  susceptible plants of this cross gave only susceptible offspring in  $F_3$ , while of the 57 resistant  $F_2$  plants studied, 16 proved to be homozygous and 41 heterozygous. The progeny of the 41 heterozygotes included 1,117 plants, of which 822 were classified as resistant and 295 as susceptible. These results indicate that in the varieties used, rust resistance is determined by a single factor difference and that resistance is dominant and susceptibility recessive. In these crosses the character of resistance to the strain of stem rust used, seems to be inherited independently of other observed characters. (Cooperative investigations by Kansas Agricultural Experiment Station and Office of Cereal Investigations, U. S. Department of Agriculture.)

*The inheritance of resistance to several biologic forms of Puccinia graminis tritici in a cross between Kanred and Marquis wheats.* OLAF S. AAMODT.

A study was made of the inheritance of resistance to several biologic forms of *Puccinia graminis tritici* in a cross between two varieties of *Triticum vulgare*. Kanred, a winter wheat, which is immune from several biologic forms to which Marquis, the spring wheat parent, is susceptible. Observations were made at University Farm, St. Paul, Minnesota, in a field in which a heavy epidemic had been induced by spraying frequently with a water suspension of urediniospores of several biologic forms. As both parents were susceptible to some of these forms, all of the  $F_2$  plants were heavily rusted in the field.  $F_2$  seedlings were then inoculated in the greenhouse with one of the biologic forms from which Kanred is immune and to which Marquis is susceptible. The plants were either immune or completely susceptible. There were no intermediates. Immunity was dominant. Inoculation experiments indicate that apparently a single factor determines the reaction to several biologic forms. Families homozygous for spring character and rust resistance were obtained in the  $F_3$  generation. (Cooperative investigations by the Minnesota Agricultural Experiment Station and the Office of Cereal Investigations, U. S. Department of Agriculture.)

*Correlated inheritance in wheat of winter-spring habit of growth and rust resistance.* OLAF S. AAMODT.

This study is one of the steps toward the production of a rust-resistant spring wheat. The parental varieties, Kanred and Marquis, belong to *Triticum vulgare*. Kanred, a winter wheat, is resistant to several biologic forms of *Puccinia graminis tritici* (Ericks. & Henn.) to which Marquis, a spring wheat, is susceptible. At University Farm, St. Paul, Minnesota, the Kanred when sown in the spring produces late in the season only an occasional head which fails to set seed. The  $F_2$  Kanred-Marquis cross was sown in the spring and the plants were placed in nine groups, according to time of heading. Seven of these groups set seed and were tested in  $F_3$ . All individuals of the earlier-heading  $F_2$  group bred true for spring habit of growth. In the six other groups in  $F_2$ , the percentage of spring plants was in direct relation to the time heading of the  $F_2$  group.  $F_3$  seedlings of each  $F_2$  group were inoculated in the greenhouse with a single known biologic form of rust. The segregation approximated a ratio of 3 resistant to one sus-

ceptible plant. The ratio of resistant to susceptible plants was approximately the same for all heading periods. Preliminary tests indicate that the reaction to several biologic forms was inherited as a single genetic factor. (Cooperative investigations between the Office of Cereal Investigations, U. S. Department of Agriculture, and the Department of Agriculture of the University of Minnesota.)

*Progress of the barberry eradication campaign.* F. E. KEMPTON.

The cooperative campaign for barberry eradication, conducted in Colorado, Illinois, Indiana, Iowa, Michigan, Minnesota, Montana, Nebraska, North Dakota, Ohio, South Dakota, Wisconsin, and Wyoming, by the United States Office of Cereal Investigations has advanced through its fourth field season. During 1918 an organization was formed, a wide-spread campaign of publicity and education conducted, and surveys to locate bushes were begun. In 1919 the city and village surveys were almost completed and a systematic farm-to-farm survey completed in about 90 counties. In 1920 a resurvey of cities and villages was conducted and the farm-to-farm survey completed in 88 more counties. In 1921 the farm-to-farm survey, with resurvey of included cities and villages, was completed in 142 counties. Of these, 23 counties were surveyed on funds furnished by the State of Minnesota. Investigations were begun on chemical methods of eradicating both mature bushes and seedlings. From April 1, 1918, to October 31, 1921, all states in the eradication area provided themselves with laws compelling barberry eradication; almost all cities, towns, and villages therein were surveyed; and an area of approximately 321 counties was covered in the farm-to-farm survey, with necessary resurveys in a portion of these counties. The original survey is completed in Montana, Colorado, and Wyoming. A total of 5,601,257 bushes was located on 49,715 properties. Of these 4,418,738 bushes were removed from 45,036 properties. Of the 1,182,519 bushes remaining on 4,679 properties, about 1,000,000 are escaped bushes, most of which are under 18 inches in height on 3 large escaped areas in Wisconsin.

*Rye resistant to leaf rust, Puccinia dispersa.* E. B. MAINS AND C. E. LEIGHTY.

Seed from a volunteer plant of rye in 1920 produced plants showing different degrees of susceptibility to the leaf rust of rye when tested in the greenhouse. Heads from four of these plants, two showing a high degree of resistance and two showing a high degree of susceptibility, were bagged in pairs in various combinations, and from the seed thus obtained plants have been grown which when inoculated with *P. dispersa*, show degrees of susceptibility from practically complete immunity to a very high degree of susceptibility. (Cooperative investigations by the Purdue University Agricultural Experiment Station and the Office of Cereal Investigations, U. S. Department of Agriculture.)

*Infection capabilities of crown rust of oats.* G. R. HOERNER.

Greenhouse inoculations of seedlings of grasses and varieties of cultivated oats were made with inoculum from four specimens of crown rust on oats collected at Lynchburg, Virginia, St. Paul, Minnesota, San Diego, California, and Tallulah, Louisiana. A summary of the work showed that there seemed to be slight differences in infection capabilities between the four specimens of the rust used in the experiments, though from the evidence at hand, no conclusions were drawn that there were a number of common hosts for *Puccinia coronata* Cda. and *P. graminis avenae* Eriks. and Henn., namely, a varying number of species of the following genera: *Alopecurus*, *Anthoxanthum*, *Arrhenatherum*, *Avena*, *Bromus*, *Dactylis*, *Elymus*, *Festuca*, *Holcus*, *Hordeum*, *Lolium* and *Phleum*; that under greenhouse conditions the crown rust of oats was capable of in-

fecting a varying number of species of the following genera: *Agropyron*, *Alopecurus*, *Anthoxanthum*, *Arrhenatherum*, *Avena*, *Bromus*, *Dactylis*, *Elymus*, *Festuca*, *Holcus*, *Hordeum*, *Hystrix*, *Lolium*, and *Phleum*. The work here reported was carried on while a graduate student at the University of Minnesota, 1916-1918. A detailed paper is in the source of preparation.

*"Black point" of wheat.* NEVADA S. EVANS.

Observations during the past year have shown that comparatively high percentage of the kernels of durum wheats of the Upper Mississippi Valley are often partly or entirely discolored, especially at the embryo end. These areas are dark brown or creosote colored. Hundreds of isolations made from typically discolored kernels have given, in the majority of cases, a species of *Helminthosporium* similar to *Helminthosporium sativum* P. K. & B. For example, in one series of black pointed seeds (260 kernels of D 5 and Acme) 77.6 per cent of the kernels yielded this fungus, while plantings from several series of apparently healthy, plump seed from the same samples gave none of this organism. Likewise, the bran layer from discolored areas when plated gave from 85 to 100 per cent *Helminthosporium*. Discolored seeds when placed in moist chambers gave uniformly a surface sporulation of this same organism. In July, 1921, a number of isolations were made in the field at Madison, Wisconsin. Water suspensions of conidia of the fungus were applied to the heads of Acme and D5 wheat when in flower. Heads thus inoculated were covered with glassine bags for one and two days. In the cases where the inoculations were made when the conditions for infection were most favorable, abundant "black-pointed" kernels resulted in contrast to a low percentage in the controls where only water was applied.

*The sesame spot disease of rice.* G. O. OCFEMIA.

The sesame-spot disease of rice caused by *Helminthosporium oryzae*, previously reported from Japan, Java, Italy, and the Philippine Islands, was observed by Dr. W. H. Tisdale in Louisiana in 1920. An undetermined species of *Helminthosporium* also has been reported on rice in the Straits and Federated Malay States and in southern China. In Japan the disease was noted as important in 1896, caused serious damage in 1899, and later threatened the rice culture in some sections. Now it is well established in Japan and causes considerable damage when it develops in nursery beds. In Italy in 1906, Farneti concluded that *Helminthosporium oryzae* and *Piricularia oryzae*, said to be the most destructive of the rice fungi, are identical. In the Philippines in May 1918, the writer noted important seedling killing by *H. oryzae*; from 10 to 58 per cent of the seedlings of susceptible varieties were killed. The disease is most destructive on the rice seedlings, causing typical seedling blight. The leaves are also attacked, resulting in leaf blight. In 1900 Breda de Haan described the causal fungus in Java as *Helminthosporium oryzae*. A year later Miyabe and Hori in Japan independently gave the fungus the same name. The fungus is seed-borne as dormant mycelium, and infection of the seedlings results when infected kernels germinate. Subsequent sporulation results and secondary infection takes place any time during the rice growing season. There are apparently some morphological variations between the strains of *Helminthosporium* from various sources and these are being studied further. In Japan hot-water seed treatment practically prevents the disease. The Japanese also mention beneficial results from spraying. In Japan and in the Philippines certain varieties of rice are more resistant to the disease than others.

*A new leaf spot of Kentucky Blue Grass caused by an undescribed species of Helminthosporium.* CHARLES DRECHSLER.

A disease due to an undescribed species of *Helminthosporium* has been found affecting *Poa pratensis* L., in Wisconsin, Illinois, New York, Connecticut, Massachusetts, Maine, Maryland, and Virginia. It is manifested by the presence on the leaf blade of bluish black spots, usually relatively few in number and not exceeding 2 or 3 mm. in length, but occasionally becoming more abundant and considerably larger. The affected leaves wither and die prematurely, the withering beginning at the tip and proceeding toward the base. Not infrequently, especially in moist locations, the sheaths at the base of the plants are also affected, the discoloration being here, however, less intense and more generally diffused, giving rise to a condition not unlike the foot rot of wheat. The parasite is somewhat similar to *Helminthosporium sativum* P. K. and B. in producing thick-walled, dark, olivaceous spores, but differs from the latter species in the somewhat greater dimensions of the sporophores, in the spores germinating by the proliferation of germ tubes from intermediate as well as terminal segments, and in its relatively slow growth and meagre sporulation on artificial media. (Cooperative investigations by the Wisconsin Agricultural Experiment Station and the Office of Cereal Investigations, U. S. Department of Agriculture.)

*Net blotch of meadow fescue caused by an undescribed species of Helminthosporium.* CHARLES DRECHSLER.

A disease that has not hitherto been mentioned in the literature, and different from the spot-blotch reported from Iowa by Pammel, King, and Bakke, has been found affecting *Festuca elatior* at stations in Maine, Massachusetts, Connecticut, New York, Maryland, and the District of Columbia. The symptoms are very similar to those induced by *Helminthosporium teres* on barley. The newly affected green tissues show abundant brownish discoloration in irregular pattern, within which may be recognized a network of darker longitudinal and transverse linear streaks. After a considerable portion of the leaf blade has been involved, it gradually withers and dies, the withering beginning at the tip and proceeding toward the base. As the destruction of foliage continues throughout most of the season, the disease is easily the most serious parasitic trouble affecting meadow fescue in the Middle Atlantic States. The fungus responsible for the malady is a species of *Helminthosporium*, the spores of which are subhyaline to yellowish, straight, usually tapering markedly toward the apex, 1 to 7 septate, somewhat similar to those of *H. gramineum* Rabh., from which they differ, however, in shape and in mode of germination. (Office of Cereal Investigations, U. S. Department of Agriculture.)

*Experiments with Haskell's method or the so-called dry formaldehyde treatment for the prevention of oat smut.* J. E. HOWITT AND R. E. STONE.

This method has been tested by the writer for four successive years. In all, thirty-five field trials under ordinary farm conditions have been made and 2,122 bushels of oats treated. The varieties of oats treated included O. A. C. 72, Alaska, Banner, White Cluster, Mammoth Cluster, and Siberian. In 1918, 61 bushels of oats were treated; percentage of smut which developed in crop from treated seed 0, percentage of smut which developed in crop from untreated seed 6.5. In 1919, number of bushels of oats treated 630, percentage of smut in crop from treated seed 0, percentage of smut in crop from untreated seed 2.26. In 1920, number of bushels of oats treated 1,016; percentage of smut in crop from treated seed 0, percentage of smut in crop from un-

treated seed 2.36. In 1921, number of bushels of oats treated 415; percentage of smut in crop from treated seed 0, percentage of smut in crop from untreated seed 5.8. The average for the four years shows no smut in crop from treated seed and 4.23 per cent of smut in crop from untreated seed. Tests were made also to determine whether this treatment injured the vitality of the seed. In the four years experiments the average percentage of germination of treated and untreated seed was found to be exactly the same, namely 97.5 per cent. It is thus seen that the results have been uniformly satisfactory throughout the four years experiments. No injury to the vitality of the seed has resulted and the control of the smut has been almost perfect. In no case has there been more than a trace of smut in any of the fields sown with treated seed, while the amount of smut in the fields sown with untreated seed for check averaged 4.23 per cent. In some of the checks there was over 15 per cent of smut present. The advantages of this method over those which have been in general use heretofore are simplicity, rapidity and ease of application. In these experiments it was found that one hundred bushels of oats could be treated in fifty minutes by this method and that there was no waiting for the seed to dry after treatment, or danger of the grain sprouting or moulding or being swollen so that it would not run freely through the drill.

*Results of treating seed of spring wheat and oats with copper carbonate dust to prevent smut.* E. B. LAMBERT AND D. L. BAILEY.

In the spring of 1921 smutty seed of Prelude wheat and of Victory oats was treated with copper-carbonate dust (copper equivalent 20 per cent). The use of two ounces per bushel controlled the smut of oats completely and the use of as much as four ounces per bushel did not reduce the percentage of germination. Treatment of wheat with two ounces per bushel controlled bunt entirely, but in preliminary laboratory tests germination was reduced 8 to 16 per cent in different seed lots. However, the plots which were planted with this seed yielded more than did the checks. Subsequent tests demonstrated that treatment of wheat with two to ten ounces of the copper-carbonate dust per bushel did not reduce the viability of the seed when planted in soil in the greenhouse. Many tests were made on spring wheat with concentrated formaldehyde and while the seed often was not injured, germination of some seed lots was reduced as much as 60 per cent. The amount of injury increased when the wheat was not planted immediately after treatment. The percentage of injury from the standard sprinkle treatment with formaldehyde ranged from 0 to 42. Both formaldehyde treatments were safe and effective for oats. The table shows the percentage of smut and the percentage of seed germination in the experiments made in 1921.

Treatment	WHEAT		OATS	
	Per cent decrease in germination	Per cent * bunt	Per cent decrease in germination	Per cent smut
Check		18.5		13
Concentrated				
Formaldehyde (50-50)	33-62	2.1	0	3.0
Standard sprinkle (1-320)	21-42	1.4	0	0.2
Copper-carbonate dust (2-4 oz.)	See text	0	0	0

\* Average of several tests.

*Potato tipburn in northeastern Maine.* DONALD FOLSOM AND E. S. SCHULTZ.

Tipburn was observed in Aroostook County during the last three summers, two of

of which were hot and dry, somewhat like mid-western summers. *Empoasca mali* was scarce, only one specimen being found in 1921 by the resident Maine Station entomologist. The hopperburn type was not seen. The sunscald type usually appeared within two to three hours of wind and bright sunlight, following several days of cloudiness and rain. The hot and dry weather type, aggravated by early blight and flea beetles and possibly other conditions which reduced the general vigor of the plant, appeared on check plots and commercial fields that had no copper spray applied, with late blight absent because of weather conditions. Furthermore, this type was severe in fields and plots sprayed well with Bordeaux mixture, and on plots, rows, hill lots, tuber units, and hills that were affected with leaf-roll, mosaic, and related diseases. This was true for certain of such hills planted and grown under cages containing no insects. This type of tipburn sometimes spreads throughout a hill in association with other signs of certain degeneration diseases. Nearby healthy controls were free from tipburn or nearly so. It seems possible that hopperburn, especially the systemic type reported from the Middle West, may sometimes be involved with mosaic and other degeneration diseases, of which the typical symptoms are modified by the climatic conditions that favor leaf hoppers.

*Leaf hopper injury of potatoes.* J. G. LEACH.

During the summer of 1921 a hitherto undescribed pathological condition of the potato plant was observed in Minnesota. Affected plants were characterized by a pronounced shortening of the leaf petioles, with the consequent crowding of the leaflets. The petioles and midveins of the leaflets also were much shorter than normal and the tips curved sharply downward and backward towards the petiole. At the same time, the margins of the leaflets were folded upward along the mid-vein. The potato leaf hopper (*Empoasca mali*) was very abundant and was constantly associated with the disease. By placing a number of the insects collected from affected plants on normal, healthy plants grown under cages, it was proved experimentally that the leaf hoppers were responsible for the disease. All plants so treated developed typical symptoms within seven days, while all check plants remained normal. Sufficient data have not been obtained to justify conclusions as to the nature of the disease and its relation to hopperburn caused by the same insect. The condition was very prevalent in Minnesota in 1921 and was undoubtedly responsible for considerable reduction in yield.

*"Skin spot": a stage of powdery scab.* MICHAEL SHAPOVALOV.

The "skin-spot" disease of Irish potato tubers has been reported from England and Canada, and as "pustulefaule" from Germany. Its peculiar characteristics are identical with those of the immature or closed-sorus stage of powdery scab. The spots have the same external appearance and the dead tissues a typical chocolate-brown or olive-brown color. Sections show a characteristic tapering spread of the infection between the cells. Fungus hyphae are sometimes very few and sometimes entirely absent. Various authors have attributed this disease either to different hyphomycetous fungi or to non-parasitic causes. This variance of opinion is due to the fact that the conclusions were based principally on cultural work. The writer's isolations from material obtained from various countries show that the fungi invading the "skin-spot" pustules vary with the locality. The progeny of some *Spongospora*-infected English potatoes planted in Pennsylvania developed "skin spot" in the absence of *Oospora pustulans*. Owen believed that in one case positive results were obtained from artificial inoculations of healthy tubers with *O. pustulans*, but the conditions of the experi-

ment do not warrant this conclusion. Hyphomycetes infesting the "skin-spot" pustules should be regarded as secondary invaders.

*Progress notes on potato wart disease investigations.* FREEMAN WEISS AND C. R. ORTON.

The season of 1921 was unfavorable for growth of potatoes in many parts of Pennsylvania, owing to deficient precipitation and high temperatures, particularly in June. Influence of untoward climatic conditions upon infection by *Synchytrium endobioticum* was exerted both directly upon the pathogen and indirectly through effect on the host which made a less succulent and susceptible type of growth. Indirect effect seems to be of greatest importance. Infection of highly susceptible varieties occurred in June during height of growth, but in general both infection and development of tumors were retarded by dry soil or high temperature. In controlled soil-temperature experiments infection occurred at 22°C., which is above optimum for growth of potato. Seven additional varieties of American potatoes are provisionally classed as immune, making 34 in all out of 103 varieties tested, but the number of types of immune potatoes remains the same, namely, McCormick, Green Mountain, Cobbler, Spaulding Rose, Ehnola, and Burbank, while the Rural New Yorker, Early Ohio, Early Rose, Triumph, Early Michigan, Pearl, and Up-to-Date types are susceptible. Eggplant, cayenne and pimento peppers, petunia, tobacco, *Datura* sp., *Solanum integrifolium*, and *S. carolinense* are not susceptible to wart disease. *S. nigrum* and *S. dulcamara* have never been found infected in America. Potato and tomato remain the only demonstrated American hosts. Immunity to wart disease is not affected by presence of leaf-roll or mosaic in the stock.

*Leak, a serious transit disease of potatoes.* GEO. K. K. LINK.

Field and market observations made during the past few years seem to indicate that leak is virtually coextensive with the potato crop of the United States, and that it is a serious transit and storage disease. In the terminal markets it has been noted in potatoes from New Jersey, New York, North Carolina, Louisiana, California, Washington, Idaho, Montana, Wyoming, Colorado, Nebraska, and Minnesota. The heaviest losses have been observed in Rurals shipped out of Idaho during the hot weather of August and September. The disease seems to occur in potatoes from other sections if the crop is dug and moved during warm weather. Potato men are reluctant to store early potatoes because "they do not keep." One of the reasons for this situation is the menace of leak in both early and late potatoes if dug and stored during warm weather. During September and October of 1921, the losses in Rurals and Burbanks in Idaho storage houses were heavy. It has been demonstrated by isolations and inoculations that most cases of leak are caused by Pythium-like fungi. During the four years only four cases of leak due to *Rhizopus* spp. and two due to *Mucor* spp. were found. Leak has been produced experimentally with the *Rhizopus* species, but not with the species of *Mucor*.

*Further experiments with inoculated and uninoculated sulfur for the control of potato scab.*  
WM. H. MARTIN.

Fifteen field experiments were conducted with sulfur in 1921. All resulted in a considerable reduction in the number of unsalable scabby potatoes. In six experiments inoculated and uninoculated sulfur was applied at the rate of 600 pounds per acre. The average number of clean tubers on the untreated plots was 8.9 per cent as compared with 33.5 per cent on the plots receiving 600 pounds of uninoculated sulfur and 50.9 per cent for those treated with a similar amount of inoculated sulfur. In three tests where

inoculated and uninoculated sulfur was applied at the rate of 300 and 600 pounds per acre the 300 pound application of inoculated sulfur showed a reduction of 45.7 per cent in the number of unsalable scabby tubers as compared with 39.2 per cent recorded for the plots treated with 600 pounds of uninoculated sulfur.

*Additions of formalin to maintain the concentration uniform with direct steam heat in the hot formaldehyde treatment of potatoes.* F. M. BLODGETT AND F. R. PERRY.

Nine thousand bushels of potatoes were treated by the hot formaldehyde method of Melhus, using steam discharged directly into the solution for heating. The concentration of the solution was determined at frequent intervals throughout the process. A tank containing about 580 gallons was used. By comparing our results with those of Melhus, as reported in Iowa Research Bulletin 59, it seems evident that the amount of formalin to be added after each fifty bushels treated to maintain the concentration uniform is not proportional to the amount of solution to be heated as suggested by Melhus. It was approximately the same for the 580 gallon tank as for the 200 gallon tank used by Melhus. If the level of solution in the tank is kept constant by condensing steam and addition of water as required, the amount of formalin to be added per bushel would appear to be approximately the amount carried off on a bushel of potatoes, regardless of the size of the tank or the method of heating. In our tests the addition of nine-tenths of a pint per fifty bushels treated seemed sufficient to maintain a concentration of 3.6 grams of formalin per liter.

*Potato-seed treatments in western states.* H. G. MACMILLAN.

Continued observations and experiments have demonstrated that no standard or uniform potato seed treatment with mercuric chloride can be relied upon to give beneficial results upon certain types of alkaline soils. On some soils treatment results in positive harm to the seed potatoes as compared with untreated control plots. Treatments have to be worked out and modified to meet local soil and water conditions. Formaldehyde is non-effective against common scab under irrigation where mismanagement in the use of water may cause an excess of soil moisture for an extended period at any time during the early stages of tuber development. Steadily growing plants, either treated or untreated, maintained free from excesses of drought or moisture appear to escape disease a longer time than where improper application of water has occurred.

*Yellow dwarf of potatoes.* M. F. BARRUS AND CHARLES CHUPP.

A hitherto undescribed disease of potatoes, called "yellow dwarf" because of its effect on the vines, has been observed in New York State. Not only are affected plants dwarfed and the foliage yellowed, but there is a necrosis of the pith and cortical cells in the vicinity of the upper nodes of the stalk. Death of affected stalks takes place from the top downward, beginning with terminal and upper axillary shoots. Tubers from affected plants are usually small, irregular, more or less sessile, brittle, and often badly cracked. There is considerable internal discoloration in the form of rusty brown specks throughout the outer medullary tissue, often extending to the bud end and but rarely to the stem end of the tuber. The number of discolored areas increases with the age of the tuber. A dry rot from the stem end, which finally involves the entire tuber, occurs on badly affected tubers. Even those otherwise apparently healthy can be detected by the more prominent lenticels. The disease affects at least eighteen varieties of potatoes and no variety has been found resistant. The agency causing the disease has not been determined. Infection evidently takes place from the soil and from infected tubers capable of producing plants.



*The correlation of foliage-degeneration diseases of the Irish potato with variations of the tuber and sprout.* ALFRED H. GILBERT.

The results of studies made at the Vermont Experiment Station during the years 1919-1921 are summarized as follows:—

1. Tubers with spindling sprouts invariably produced either leaf roll plants or plants possessing both mosaic and leaf roll symptoms.
2. Spindliness of sprout was often correlated also with net-necrosis. Eyes in or near necrotic tissue produced spindling sprouts, while other eyes from non-necrotic portions of the same tuber produced sprouts apparently normal. No disease-free plants, however, were secured from tubers either partially or entirely necrotic.
3. Every net necrosis tuber produced plants showing typical and advanced leaf-roll, but not all leaf-roll plants were from net-necrosis tubers.
4. Well marked symptoms of both mosaic and leaf-roll occurring simultaneously in the same plant have been observed in a number of instances.
5. Tuber unit series of plants from leaf-roll tubers showed gradual decrease in size and vigor of plants produced from middle and stem-end buds as compared with those grown from blossom-end buds.
6. No plants free from disease have been secured from any eyes of leaf-roll, mosaic, or net-necrosis tubers.
7. Tubers with apparently normal sprouts may produce plants showing at least mild mosaic symptoms.

*The relation of time and temperature to the killing of potatoes and potato mosaic virus.*  
F. M. BLODGETT.

Bliss Triumph potatoes from plants affected with mosaic were treated in water at temperatures from 35 to 80° C. for the purpose of determining, if possible, the relation of time and temperature to the killing of the potatoes and the mosaic virus. It was found that when the logarithm of the time of killing potatoes was plotted against the temperature a straight line resulted so that the relation of time to temperature for this reaction may be approximately expressed by the formula  $\text{Log}_{10} t = -.107X + 7$  in which  $t$  is time in minutes and  $X$  is temperature in degrees centigrade. All potatoes that sprouted after this treatment were planted and gave rise to plants affected with mosaic. This would indicate that at least in the range of temperature used the time necessary for the killing of the mosaic virus is longer than for the killing of the potatoes.

*Testing seed potatoes for mosaic and leaf roll—II.* F. M. BLODGETT, KARL FERNOW, and F. R. PERRY.

The testing of seed potatoes as reported last year, by growing one piece from each potato in the greenhouse, was continued. The outstanding result of this year was that practically all potatoes thus indexed as being affected with mosaic failed to show symptoms of the disease in the field under the conditions prevailing in New York State this year. These were planted in three different counties in different parts of the state. This result would seem to indicate the general unreliability of counts made on mosaic and the impossibility of removing mosaic plants by roguing under such conditions. Tubers indexed as leaf roll grew sprouts generally thinner than healthy potatoes, but not always of the extreme spindling sprout type. From two lots of seed containing about 50 per cent leaf roll, all but three per cent were removed by these methods. Tubers were also indexed by growing one piece from each potato as an early crop in the field previous to planting the main crop. This was only partially successful under the

conditions this year. One-fourth to three-fourths of the mosaic in different tests and about nine-tenths of the leaf roll in one test were removed by this method.

*Transmission of potato streak.* E. S. SCHULTZ and DONALD FOLSOM.

Preliminary observations in northeastern Maine indicate that streak is closely related to mosaic and similar diseases of the Irish potato, being frequently associated with them in the field, having initial late-season symptoms in new leaves only, usually spreading to all connected parts of a hill, and spreading to other hills with the production either of late-season symptoms the same year or of tuber-transmitted early-season symptoms appearing the following year. In 1921 juice from a streak plant applied to 20 mutilated Green Mountain and Irish Cobbler plants caused infection in 19, with typical symptoms appearing in some in 12 days. Sixty control hills in the same tuber-units, from quartered tubers, remained healthy. In similar series of tuber-units subjected to control inoculations, juice from curly-dwarf Carman No. 3 plants produced mosaic-dwarf infection, while juice from mosaic Bliss Triumph plants caused mosaic symptoms only in the upper leaves of caged hills. The yield of the inoculated plants indicated that the rareness of streak and mosaic-dwarf in commercial fields of north-eastern Maine may be due to self-elimination.

*Experiments with winter blight or streak of tomatoes.* R. E. STONE and J. E. HOWITT.

This is a very common disease in tomatoes grown under glass in Ontario, often causing serious losses and in some instances making the production of a profitable winter crop of tomatoes impossible. The disease sometimes occurs in fields, especially in those very heavily manured. The name winter blight has been given to this disease because it is especially troublesome in the winter crop of tomatoes. It is called streak by the growers on account of the characteristic dead, brown, shrunken lesions appearing on the stems. Experimental work was begun on this disease in 1914 and has been continued every year since. Some of the experiments were carried on in the college greenhouses, but most of them were conducted in large commercial greenhouses. A preliminary report on this disease was published in *Phytopathology*, Volume 6, No. 2, 1916. The results of the experiments conducted up to that time lead the writers to conclude that no pathogene was responsible for this disease, but that it was the result of soil conditions. Subsequent work has supported these preliminary conclusions. During the past five years almost uniformly satisfactory results have been obtained in the control of this disease by the addition of phosphoric acid and potassium to the soil. In many cases it has been found possible to cause tomato plants to outgrow the winter blight by the application of these fertilizers to the soil. Such treatment has resulted in the saving of large commercial crops of tomatoes in Ontario.

*Overwintering of tomato mosaic.* MAX W. GARDNER and JAMES B. KENDRICK.

Over twenty thousand tomato plants were grown from seed from mosaic plants, but no evidence of seed transmission of mosaic was obtained. Mosaic has been found in old tomato fields on the perennial solanaceous weeds *Physalis subglabrata*, *P. virginiana*, *P. heterophylla*, and *Solanum carolinense* and mosaic has been transmitted from each to tomatoes. Rootstocks of mosaic *P. subglabrata* transplanted to a garden in August, 1920, produced mosaic shoots the next spring at an earlier date than tomatoes are transplanted. This weed is very prevalent in central Indiana. Examination of *Physalis* in nine fields previously in tomatoes showed that a high percentage of the plants showed mosaic the next year and the second year after the tomatoes. *Physalis* was observed in

65 out of 81 tomato fields and mosaic was noted on *Physalis* in 35 of these fields and on both *Physalis* and tomatoes in 29 fields. Tomato mosaic was noted in 60 fields, in 48 of which *Physalis* occurred. As new fields are used for tomatoes, the reservoir of mosaic in the perennial weed flora will increase each year. The most destructive type of tomato mosaic seems to be of plant-bed origin and the presence of *Physalis* near plant-beds is especially dangerous.

*Further studies on mosaic — I.* B. T. DICKSON.

Juice inoculations from mosaic-diseased *Trifolium pratense* were successful in from ten to fifteen days on *T. pratense* 12/23, *T. repens* 3/9, *T. hybridum* 15/32, *T. incarnatum* 2/5, *Medicago lupulina* 3/8, *Melilotus alba* 0/7, and *M. officinalis* 0/5. Using *Macrosiphum pisi* Kalt. (det. by Dr. E. M. Duporte) as the agent of inoculation from mosaic-diseased red clover plants, successful results were *T. pratense* 18/27, *T. hybridum* 15/26, *M. lupulina* 5/8, *T. repens* 4/7, *T. incarnatum* 3/5, *M. alba* 0/8 and *M. officinalis* 0/7. Using *Macrosiphum pisi* Kalt. from healthy *T. pratense* on 7 healthy *T. pratense* plants, no mosaic showed at the end of two months. Tests were conducted from February to September, 1921, in the greenhouse. N. B. X/Y indicates X successful out of Y inoculated.

*Further studies on mosaic — II.* B. T. DICKSON AND G. P. McROSTIE.

At Macdonald College out of 1075 *Trifolium pratense* plants 47 per cent showed mosaic in Sept. 1920. The same plants on June 30, 1921, showed 91 per cent mosaic, 6.7 per cent doubtful, and 2.1 per cent healthy. *Macrosiphum pisi* Kalt. was abundant during the early summer this year. Twenty-two mosaic-diseased plants yielded 1,443 seeds or 65 seeds per plant. Twenty-two healthy plants yielded 10,566 seeds, or 484.8 seeds per plant. Inheritance tests in the greenhouse in sterilized soil of the 1,443 seeds from diseased plants gave germination 186, of which 125 were healthy, 37 doubtful, and 24 were diseased within 10 days after germination. Commercial seed from St. Rosalie sown in the field October, after frosts had checked the remaining aphids, showed 5 distinctly diseased plants and several doubtful ones out of approximately 10,000 plants at the time of snowfall November 10. With commercial alsike seed in the greenhouse, of 210 seeds planted, 34 germinated, of which 31 were healthy, 2 doubtful, and 1 mosaic-diseased 10 days after germination.

*Mosaic studies.* I. E. MELHUS.

*Physalis longifolia*, a perennial *Solanum*, has again been found to carry mosaic over winter and transmit it to tomatoes, peppers, potatoes, *Petunia*, and several wild species. The mottling and crinkling characteristic of mosaic are masked on egg plant (*Solanum Melongena*) in the greenhouse on plants that have passed the seedling stage. The only evidence of an abnormal condition is its smaller size and the infectiousness of its juices on tomatoes, where typical mosaic develops. Stem tissues of mosaic-infected potatoes and tomatoes have been found to contain certain bacteria and other organisms not found in healthy stem tissue. The presence of organisms in mosaic-infected tissue is often markedly constant and probably largely responsible for the marked dwarfing.

*Notes on cucurbit mosaic.* S. P. DOOLITTLE AND M. N. WALKER.

Continued studies of cucurbit mosaic show that the milkweed, *Asclepias syriaca*, is an important agency in overwintering the disease. Practically all of the mosaic milkweeds found have occurred in the neighborhood of cucumber fields and were evident sources of

primary infection. The milkweed probably becomes infected from adjacent cucumber plants and being perennial acts as a center of infection in succeeding seasons. Cross-inoculation experiments indicate a possible transmission of cucurbit mosaic to the potato. Caged potato plants, on which mosaic cucumber aphids were colonized, developed mosaic symptoms in three out of five cases. Aphids from all of these potato plants, regardless of the presence of mosaic symptoms, were transferred to healthy cucumber plants after four to six weeks and nine out of twenty-one plants inoculated developed mosaic. Aphids from the inoculated potato plants of normal appearance produced mosaic, as well as those from plants showing mosaic symptoms. Potato plants on which healthy aphids were colonized made a normal growth, and aphids from these plants failed to produce mosaic on the cucumber. No mosaic was found on any uncaged potato plants in the plot. Mosaic cucumber aphids transferred to healthy pokeweed plants produced symptoms of mosaic in three out of five cases.

*Cucumber black rot caused by *Mycosphaerella citrullina*.* FRED MEIER, CHARLES DRECHSLER AND EMERY EDDY.

Cucumbers shipped from Florida on arrival at the New York market have often been found considerably damaged by various decays. Among the more important of these is a rot attributable to *Mycosphaerella citrullina* (Smith) Gross., a fungus known as occasionally destructive to greenhouse muskmelons and to watermelons in the Southeast, but not hitherto reported on cucumbers. Affected cucumbers develop water-soaked areas that, although enlarging less rapidly than those occasioned by *Rhizopus* sp., may attain considerable size during the time required for transportation. Cucumbers inoculated with the fungus at Wauchula, Florida, on arrival in New York City, were found badly decayed and in many instances bore an abundance of pycnidia and perithecia. The decay is readily distinguished from similar troubles due to other organisms by the darker color of affected portions and firmer texture of diseased tissues, the firmness being due to the rather compact mycelial development immediately beneath the epidermis preparatory to production of the very numerous imperfect and ascigerous fruiting bodies. (Cooperative investigations Bureau of Plant Industry and Bureau of Markets Inspection Service.)

*Further notes on the occurrence of cabbage black leg.* J. C. WALKER AND W. B. TISDALE.

The importance of rainfall in the development of black leg, previously emphasized (Phytopath. 10: 64), has been further studied with special reference to occurrence of the disease in the Puget Sound seed growing region. Nearly simultaneous plantings of infected seed at Madison, Wis., and LaConner, Wash., May 2 and May 7, 1921, were examined at transplanting time on July 7 and July 23, respectively. At Madison 65 per cent of the plants showed leaf or stem lesions and at LaConner 3 per cent. Comparative rainfall records for May, June, and July, respectively, at Madison were 5.13, 3.52, and 2.46 inches; and at LaConner, 1.89, 1.90, and 0.00. Thus with the rainfall somewhat above normal at LaConner only a very slight development of black leg occurred and with the dry weather, which normally continues through July and August, it is doubtful whether this amount of disease would have survived after transplanting. Black leg has never been reported from this region. Moreover, one seed field at LaConner, grown from stock seed which developed 75 per cent black leg under Wisconsin conditions, showed no signs whatever of the disease. These facts, in view of the limitations of seed treatment, suggest the feasibility of growing black-leg-free seed in the Puget Sound region.

*Observations on the spore content of the upper air.* E. C. STAKMAN, A. W. HENRY, W. N. CHRISTOPHER, AND G. C. CURRAN.

As a part of the rust epidemiology studies made by the Office of Cereal Investigations of the United States Department of Agriculture during the spring and summer of 1921, aeroplanes were used to ascertain the spore content of the upper air. Ordinary microscopic slides, smeared with vaseline, were placed in a mechanical spore trap which could be manipulated from the cockpit in such a way that it was possible to expose the slides one at a time for as long a period as desired, at various altitudes and at widely separated points. In addition to rust spores, numerous spores of *Helminthosporium*, *Alternaria*, *Cladosporium*, *Cephalothecium*, and *Ustilago* were caught at elevations up to ten thousand feet. Some were caught at higher altitudes. Many pollen grains also were caught. *Alternaria* spores which had been obtained at eleven thousand feet germinated readily. The germination tests with the rust spores were inconclusive.

*Investigations on Puccinia helianthi Schw.* D. L. BAILEY.

The uredinial stage develops six to eight days after inoculation. The age of the host does not influence the degree of infection. Infection follows six hours' incubation. Uredinia will not develop below 10° C., but the mycelium will remain dormant in infected leaves for at least a month at this temperature and will develop subsequently. Optimum temperature for urediniospore germination is about 18° C. The germ tubes enter through the stomata. Teliospores germinate either immediately or after a rest period. The promycelium typically produces sporidia, but it may branch, or the cells may produce germ tubes. The aecial stage is usually produced, although it sometimes is omitted. Aeciospores remain viable about three weeks. Penetration stomatal. In 1920 urediniospores from *Helianthus subrhomboides* Rydb. and urediniospores and heliospores from *H. scaberrimus* Ell., *H. Maximiliani* Schrad. and wild *H. annuus* L. heavily infected cultivated sunflower. The same cultures on eight horticultural and cultivated varieties showed no specialization. This year Mammoth Russian shows distinct resistance to collections of rust from *H. grosseserratus* Martens, *H. Maximiliani* Schrad., and two cultures from *H. hirsutus* Raf. One collection from *H. tuberosus* L. gave two types of infection, one very susceptible and one resistant. Apparently, therefore, there are biologic forms.

*Studies on Septoria diseases of cereals and certain grasses.* GEORGE F. WEBER.

During the past two years, investigations have been conducted at the University of Wisconsin on the *Septoria* diseases of cereals and certain grasses and it has been found that there are a number of closely related species on these hosts. Morphologically these species differ only slightly. *Septoria tritici* Desm. and *S. glumarum* Pass., which occur on wheat and rye, differ distinctly morphologically. Both species cross-infect readily on these hosts and both species also infect *Poa pratensis*. No other host has been found susceptible to either species. *S. secalis* Prill. and Delacr. has been found on rye. It infects rye readily, but no other host has been found susceptible. The species on barley seems definitely to be *S. passerinii* Sacc. It infects various barleys readily, but no other host has been found susceptible. A species on oats seems definitely to be *S. avenae* Frank. It infects various species and varieties of oats, cultivated and wild, but no other host has been found susceptible. *S. agropyri* E. and E. infects *Agropyron repens* readily, but does not infect cereals. A form on *Poa pratensis*, apparently *S. graminum* Desm., infects *Poa pratensis* readily, but does not infect cereals.

*Relation of temperature, soil moisture, and oxygen to the germination of the spore of Ustilago avenae.* EDITH SEYMOUR JONES.

In connection with investigations of the physiological factors affecting the infection of certain species of Avena by *Ustilago avenae* (Pers.) Jens., certain of these factors, temperature, soil moisture, and oxygen were studied separately in their relation to the germination of the spore of the fungus. With regard to temperature, the cardinal points were found to be: Minimum about 5° C., optimum 15 to 28° C, and maximum ranging between 31 and 34° C. Experiments with soil moisture were conducted by duplicating as closely as possible the conditions under which germination takes place in the field, using three different temperatures, 10 to 13°, 20 to 24°, and 30 to 33° C. When the moisture in the soil at these temperatures reached 60 per cent of the water-holding capacity, germination percentages decreased slightly and were greatly reduced at 80 per cent. The supposition that a lack of oxygen in this soil of high water content might perhaps be responsible for this marked decrease led to attempts to germinate spores in the absence of oxygen. These experiments demonstrated for this fungus the hypothesis commonly assumed, that spores will not germinate in a liquid which is not exposed to oxygen. (Cooperative investigations by the Wisconsin Agricultural Experiment Station and the Office of Cereal Investigations, U. S. Department of Agriculture.)

*Fusaria of wheat and corn.* C. D. SHERBAKOFF.

Recently the writer made cultural examination of a number of samples of wheat and corn seed. These examinations, and the cultures received from Kurtzweil, show that in Tennessee, as in other states, the most common *Fusarium* of wheat is *F. graminearum* Schwabe (*Gibberella Saubinetii*) and of corn, *F. moniliforme* Sheldon. Several times corn seed yielded cultures of *Fusaria* of the *Elegans* section. Only in a few instances *F. graminearum* was isolated from corn seed; but, the perithecial stage of this fungus was often found on corn stalks. *F. moniliforme* was not common on wheat seed. Among the *Fusaria* answering Sheldon's description of *F. moniliforme* are several that differ from each other in more than one important character and are thus apparently different organisms. For this reason and because none of the previously established sections of the genus *Fusarium* fits the characters of these corn fungi, a new section, *Moniliform* with characters as follows, is proposed: Macroconidia of intermediate *Roseum-Elegans* type, with very thin walls, mostly three-septate; microconidia also in chains; no chlamydospores; color of substratum from none to violet.

*The effect of Cronartium ribicola upon Ribes.* L. H. PENNINGTON.

Four seasons' observations upon certain *Ribes* bushes in localities where *Cronartium ribicola* is abundant have shown that the rust often affects seriously some species of *Ribes*. Individual bushes of *Ribes Cynosbati*, *R. rotundifolium*, and *R. glandulosum* died after early defoliation for three successive seasons. In certain restricted localities, where there was a heavy infection of the pines, the *Ribes* have all been killed. Sprouts from the base of dying bushes did not make any considerable growth, for they also were defoliated and died within two to three years. Early defoliation and the subsequent death of *Ribes* are both factors in the control of the disease upon pine. Early defoliation greatly reduces or prevents entirely the formation of telia, and the death of the *Ribes* may occur before all the pines in that location have become infected. Under certain conditions, where there has been a heavy seeding of pine, many of them may be infected and yet enough of them escape to insure a good stand. The destruction of *Ribes* by the rust has led to error in determining the distance that infection has spread from *Ribes* to pine.

*Notes on Cronartium ribicola.* PERLEY SPAULDING.

Infected leaves of *Ribes nigrum* and *R. cynosbati* almost invariably roll the edges upward when drying so that the entire lower surface is exposed for a maximum freeing of the sporidia. In September bushes of *R. cynosbati* with a full complement of leaves bore none of the fungus, while bushes that were infected had relatively few leaves. Telia collected on dead or dying leaves (even of *R. nigrum*) or on dead spots on living leaves, in warm weather of September and October, did not germinate when collected; but those on active, green leaves from the same bushes germinated readily. Conditions in a living leaf bearing telia, which is suddenly killed, seem to favor maximum germination. Cold is known to stimulate germination of all the spores of *Cronartium ribicola*. The above observations led to the belief that heavy frost would stimulate germination and this has proven to be the case. Maximum germination has been secured on leaves exposed to and killed by frost, or which have persisted on the bush until snow fell. Such leaves have borne readily germinated telia as late as December 3 in Vermont. *Ribes nigrum* leaves collected October 11 and since then exposed to moisture out of doors but not to full sunlight are still giving good germination of the telia. When conditions are right some of the telia germinate themselves in the mosquito netting bags. This has happened only since snow was on the ground.

*A preliminary report on cross-inoculation experiments with strains of Cladosporium from stone fruits.* M. BNSAUDE AND G. W. KEITT.

Morphological studies and cross-inoculation experiments with strains of *Cladosporium* from stone fruits have been undertaken with the aim of furthering our understanding of the relations of these pathogens to their hosts and to one another. The host plants in the experiments here reported were *Amygdalus persica* L. (Elberta), *Prunus armeniaca* L. (Superba), and *P. americana* Marsh. (seedling), located in plats previously described by the junior author (Keitt, G. W. Inoculation experiments with species of *Coccomyces* from stone fruits. Jour. Agr. Res. 13: 539-569. pl. 55-59. f. 1-3. 1918. See p. 545-546). Similar trees, uninoculated, served as controls. The strains of *Cladosporium* used were taken from *A. persica* and *P. americana*. The inocula were prepared by washing cultures or host organs on which *Cladosporium* was sporulating with sterile distilled water, and were applied to leaves and twigs by means of sterile atomizers. For three days after inoculation the experimental plants were covered with a moist chamber, which has been described previously (loc. cit., p. 546-547). The outstanding results were that the fungus from *A. persica* cross-infected *P. americana* and *P. armeniaca*, and the strains from *P. americana* crossed to *A. persica* and *P. armeniaca*. The uninoculated plants developed no infection.

*Coconut bud rot in the Philippines.* OTTO A. REINKING.

Investigations in the Philippines have proved that the infectious type of coconut bud rot is caused by a specific fungus in the *Phytophthora* group. Inoculation studies conducted with bacteria seem to show conclusively that the true bud rot is not produced by these organisms. Inoculations with the *Phytophthora*, isolated from a diseased tree, in all instances caused infection through injuries and in the majority of cases caused infection of the uninjured coconuts. There are two types of bud rot in the Philippine Islands, the real infectious type caused by the *Phytophthora*, and a secondary type following some injury, such as beetle injury, and caused primarily by the invasion of bacteria in the weakened tissue. The first type accounts for the epidemics that occur principally in the chief coconut sections in Laguna Province. The second type assigns

the cause for the other scattered cases of disease found throughout the rest of the archipelago. The *Phytophthora* isolated from coconuts is similar to the *Phytophthora* producing the black rot and canker of cacao and appears to be identical with other *Phytophthoras* isolated from various other diseased hosts. Because of the prevalence and destructiveness of these *Phytophthoras*, their investigation is a field of just importance in tropical plant disease studies.

*A preliminary report on a serious twig blight of American elm.* FRED A. DETMERS.

The appearance of infected trees is described and illustrated. The causal organism from careful and repeated observational examination of dying trees is thought to be *Poronidulus conchifer* (Schw.) Murrill, a small bracket fungus of the Polyporaceae. The fungus is described in detail and illustrated. The mycelium is perennial, ramifying through the leptom and cambium and invading the sap wood, coloring the latter brown. Each year annual hymenophores are produced on dead branches, through the distribution of whose spores infection is disseminated. Premature defoliation is a symptom of infection, followed soon by the death of the shoot and later the death of the larger branches until the entire crown is involved. Fragment cultures from infected tissues and fruiting bodies of the fungus were made. A history of the outbreak in Ohio is given and attention called to the fact that infection has evidently existed for a number of years and reached its culmination in a number of large, fine trees in widely separated areas in Ohio during the past summer. A brief résumé is given of the description and naming of the fungus.

*A hybrid bean resistant to anthracnose and to mosaic.* DONALD REDDICK.

From published work of others it is known that White Imperial is resistant to strain alpha of *Colletotrichum lindemuthianum*, practically immune to strain beta, and that it is somewhat tolerant to mosaic. Robust is immune to strain alpha and susceptible to strain beta; it is immune to mosaic. From hybrids of these two varieties families of plants have been isolated which in the sixth generation (five inoculations) are immune or highly resistant to both diseases. Some of the plants are more resistant to anthracnose than either parent. In a few of the families commercial types are approximated, but commercial possibilities are not yet determined. No genetical studies have been made.

*A yeast parasitic on lima beans.* S. A. WINGARD.

Examination of lima beans from eastern Virginia, which showed numerous dark sunken areas on the cotyledons, having the appearance of bacterial origin, revealed the presence of the vegetative cells, asci, and ascospores of a yeast, a species of *Nematospora*. Some seed which had been attacked in the early stage of development were completely dried up, being no more than one-tenth normal size. The evidence strongly suggests that the yeast is parasitic and the disease appears to be of economic importance. Pure cultures have been secured and infection studies are in progress.

*Colletotrichum pisi* Pat. on garden peas. R. E. VAUGHAN AND FRED REUEL JONES.

Anthrachnose of the garden pea was first found in Ecuador by von Lagerheim and has been recently described by Hemmi in Japan. In 1912 this disease was found causing severe injury to peas in one locality in Wisconsin. In 1921 it appeared in another locality in this state as a cause of serious disease. The fungus has been cultured and in artificial inoculations, as well as in the field, found to be one of the most destructive foliage



parasites known. The similarity of the symptoms of this disease to those of the disease caused by *Ascochyta pisi* may be an explanation for the fact that it has not previously been reported in the United States.

*Spraying and dusting for the bacterial and late blights of celery in western New York.* H. W. DYE AND A. G. NEWHALL.

During the past three years equivalent amounts of 5-5-50 Bordeaux mixture and 15-85 copper-lime dust have been tested comparatively for the control of celery blights caused by *Bacterium appii* Jagger and *Septoria Petroselinæ* Desm. var. *appii* Br. and Cav. Comparative treatments were made using both power and hand machines. Applications were made at seven and ten-day intervals, beginning with the first appearance of blight. With uniform consistency throughout a considerable number of trials, almost complete reduction of blight development was obtained. The spray and dust appeared to be equally effective in controlling these blights, and both gave similar increases in yields over the checks.

*First progress report on "yellows"-resistant Golden Self-blanching celery.* G. H. COONS AND RAY NELSON.

Yellows of celery, due to *Fusarium* sp., is present in all celery-growing areas in Michigan and is reported from several other states. Certain green varieties, notably Easy Blanching, under cool conditions are tolerant to the disease, but Golden Self Blanching is so susceptible as to make its culture impossible. Seed from a plant of Golden Self Blanching, selected from a field showing practically 100 per cent loss, has been extensively tested in "sick" soil in both greenhouse and field against standard varieties of celery and in particular against pure-line strains of Golden Self Blanching. Almost complete failure of all plants except the resistant celery occurred. The resistant celery produced about 90 per cent of a crop, in spite of most severe epidemic conditions, brought about by the high soil temperatures. About 50 plants grown in the winter of 1920-21 in sick soil were brought to maturity and seed obtained. Due to extremely hot season the seed production was light. Commercial distribution of seed will therefore be delayed. The original isolation was a plant of the best Golden Self Blanching type and the progeny saved has been held to this standard.

*The bottom-rot disease of western New York lettuce.* H. W. DYE.

This most destructive disease of Boston head lettuce on New York muck land is caused by *Rhizoctonia*. Rusty lesions on the midribs and a rotting away of the leaf blades of the lower leaves is characteristic. A blackened, erect "stump" is the advanced stage of the disease. The greatest loss results on older, damp muck. The usual control measures attempted for soil-infesting organisms are ineffective or impractical. No lettuce seems to possess any resistance, though the more erect types are unaffected by bottom rot because of their disease-escaping habit. Green, erect, Romaine lettuce was hybridized with the yellow, spreading Big Boston type so affected by bottom-rot. Hybrid plants have been secured, which promise to escape bottom-rot, to possess the Big Boston color and quality, and to be but slightly more erect.

*Downy mildew: A transit disease of lettuce.* GEO. K. K. LINK.

Downy mildew of lettuce, caused by *Bremia lactucae*, has been observed in the markets in lettuce shipments from California, New York, Texas, and Washington. It causes heavy losses. At times the lesions show the typical angularity of field lesions

but generally their borders have become irregular and indefinite, indicating enlargement in transit. Observations made in California indicate that new lesions develop in packing houses, on loading platforms, and probably in the early stages of transit. However, spread and development of the disease in transit are slight, and in the markets the lesions generally appear as more or less angular, brown to black areas, with rather definitely marked outlines. Although the losses caused by these lesions are heavy, the most severe losses are due to organisms which invade the lesions and then spread to healthy tissues. Bacteria and *Alternaria* spp. are the prevalent secondary invaders. Because of these, especially the former, downy mildew is one of the most serious transit diseases of California lettuce.

*Storage rot of carrots caused by a new species of Alternaria.* FRED MEIER, CHARLES DRECHSLER, AND EMERY EDDY.

Dealers on the New York market have frequently complained of wastage due to decay of carrots in transit and storage. In addition to other and better known decays, a trouble has appeared characterized by softening and blackening of the affected tissues, beginning at any place on the surface of the root, but most frequently finding inception at the crown and extending down into the central core. On the surface of the disorganized portions, the causal organism develops as a grayish-black mat mycelium, the fuliginous hyphae of which bear an abundance of darker sporophoric branches, producing usually a single large muriformly septate spore. That the fungus is nevertheless not to be referred to the genus *Macrosporium* becomes evident on cultivation, for on media permitting protracted development a catenulate habit ultimately becomes apparent. The parasite differs markedly from *Macrosporium carotae* E. and L., the cause of leaf blight, although field observations carried out in Massachusetts and on Long Island indicate that attack by the latter fungus may predispose roots to subsequent decay in storage. (Cooperative investigations Bureau of Plant Industry and Bureau of Markets Inspection Service.)

*A new disease of asparagus.* MEL T. COOK.

During the late summer of 1921 our attention was called to the slow dying out of asparagus plants over circular areas. The plants are stunted, gradually turn brown and finally die. A careful study of the diseased plants showed brownish lesions on the part of the stems below ground and a heavy infection with *Fusarium* sp. We then recalled that from time to time for several years past, dwarfed shoots have been sent to us during the cutting season and that *Fusarium* sp. was the only organism found on them. Our studies are not complete, but all evidence up to the present time indicates that the troubles are due to *Fusarium* sp.

*Bacterial root rot of horseradish in New Jersey.* R. F. POOLE.

A bacterial root rot of horse-radish has caused losses ranging from 4 to 28 per cent on farms near Newark where this plant is cultivated. The main source of infection has been the dissemination of the disease in the storage pits. Roots carefully selected at digging time and bedded under normal conditions over winter developed from 25 to 50 per cent infection in the pits. When planted the diseased roots either failed to germinate or produced stunted plants. Good progress has been made in controlling the disease by cutting an inch from both end of seed roots and before planting discarding all that showed discoloration due to disease. Treatments with bichloride of mercury 1 to 1,000 for 15 minutes and formaldehyde 3 pints to 50 gallons for 30 minutes reduced the losses still more when the cut and selected roots were treated.

*Pathogenicity of Macrosporium parasiticum.* N. G. TEODORO.

*Macrosporium parasiticum* Thümen has generally been considered a secondary parasite to onion mildew and incapable of independently infecting the onion. In Wisconsin, where mildew seldom occurs, *M. parasiticum* is found repeatedly, apparently causing distinct lesions on leaves and seed stems of onion and often girdling the latter. Experiments were undertaken to determine the pathogenicity of the organism. Greenhouse inoculations were made with mycelium from young cultures, applied through wounds or directly to the uninjured tissue. Characteristic symptoms of the disease were produced in both cases. Inoculations in the field with mycelium and with suspension of conidia also yielded positive results. Infection occurred more readily when plants were kept moist for a short time after inoculation by means of moist chambers. Results indicate that *M. parasiticum* is capable of acting independently as an aggressive parasite. The cultural characters of the fungus are being studied in comparison with *M. porri*, which also occurs commonly on onion, and with an undescribed species of *Macrosporium* causing a bulb rot of onion (Phytopathology 11: 53).

*The decay of various vegetables and fruits by different species of Rhizopus.* L. L. HARTER AND J. L. WEIMER.

A study has been made of the susceptibility of 27 different hosts to infection by the following species of *Rhizopus*: *nigricans*, *reflexus*, *microsporus*, *arrhizus*, *tritici*, *nodosus*, *maydis*, *delemar*, *oryzae*, *artocarp*, and *chinensis*. *R. microsporus* and *chinensis* infected only a few of the hosts. The species of the intermediate temperature group, which includes *R. tritici*, *nodosus*, *maydis*, *delemar*, *oryzae*, and *arrhizus*, are more vigorous parasites under artificial conditions than *R. nigricans*, *reflexus*, *microsporus*, and *artocarp*, the representatives of the low-temperature group. *R. nigricans*, however, seems to be the predominating species causing decay of vegetables and fruits in storage and on the market. The different hosts differed in the method required to bring about infection. Beets, Irish potatoes, and those hosts apparently low in water content could be infected only by the use of the "well" method. Those hosts with a high water content could be infected by merely inserting the spores and hyphae into a wound. A wound was required for infection in the case of all the hosts, with the possible exception of the peach. Ripe peaches, with no apparent wounds, could be infected by immersing in a spore suspension.

*The control of angular leaf spot of cotton.* C. A. LUDWIG.

A method of seed treatment for preventing angular leaf spot of cotton which was devised at the South Carolina Experiment Station a few years ago was given a supplementary field test during the past season. The method consists in delinting the seed with strong sulphuric acid, washing, treating with mercuric chloride solution, washing, and drying. The treatment was found entirely satisfactory. The full account is in the hands of the editor of Phytopathology.

*Cotton wilt a seed-borne disease.* JOHN A. ELLIOTT.

Isolated wilt-infected cotton plants in otherwise healthy fields called attention to the probability that the disease was seed-borne, as has been suggested by other investigators. In 1920 a large field of isolated virgin soil was planted with acid-delinted, disinfected seed. A few cases of wilt occurred in this field. In another small isolated patch grown from disinfected seed far from the cotton region a similar occurrence of wilt was noted. A considerable quantity of seed was collected from plants which died of wilt, delinted

with concentrated sulphuric acid, strongly surface sterilized with 1: 1000 50 per cent alcohol solution of corrosive sublimate, and germinated on sterilized filter paper in petri dishes. Approximately 3 per cent of the seeds gave cultures of *Fusarium vasinfectum*. Other fungi, especially *Colletotrichum* and an unidentified *Fusarium*, were more abundant.

*Stem rot diseases of sweet potatoes in New Jersey.* MEL T. COOK AND R. F. POOLE.

The stem-rot diseases of sweet potatoes caused by *Fusarium hyperoxysporium* and *Fusarium batatis* (Wr.) are abundant in New Jersey. From 35 to 65 per cent infection has been found by splitting green vines at digging time. Infected vines frequently produce more and smaller potatoes than healthy vines. The disease is widely distributed and is severe throughout the Camden and Salem areas, being most destructive on light sandy soils. Seed selected at digging time produced slightly better sprouts than unselected seed when set on infected soils. Sprouts that become very large and hardened, due to drying of hot-bed, are very susceptible to infection after they are set in the field. Sprouts set in the field a short time after they became large enough were less susceptible. The Big Stem varieties are more resistant to stem rot than the smaller-stem varieties. The Big Stem Jersey strains developed from sports vary in resistance to the stem rot disease and they are offering an interesting comparative study of stem-rot resistance.

*The species of Rhizopus responsible for the decay of sweet potatoes under storage conditions.*

J. I. LAURITZEN AND L. L. HARTER.

*Rhizopus tritici* and *R. nigricans* are the species chiefly responsible for the decay of sweet potatoes known as soft rot, *R. tritici* at the higher temperatures and *R. nigricans* at the lower, the two overlapping between 20° C. and 30° C. Although other species are capable of causing soft rot, they do not seem to do so under the storage conditions at Washington. *R. tritici*, *R. reflexus*, and *R. artocarpi* can not compete successfully with *R. nigricans* when sweet potatoes are inoculated with any one of these organisms along with *R. nigricans*. Even though sweet potatoes are inoculated with spore suspensions of high concentration of *R. tritici*, *R. oryzae*, and *R. reflexus*, *R. nigricans* nearly always causes more decay than any of these species. *R. nigricans* causes far more decay than any or all other species; in fact it is the principal agent of decay. *R. tritici* is not normally a factor, because sweet potatoes are stored as a rule at a temperature below which it operates.

*Preliminary report on a study of the wildfire disease of tobacco.* C. M. SLAGG.

In connection with studies upon the wildfire disease of tobacco caused by *Bacterium tabacum* Wolf and Foster, morphological studies have been made of the causal organism isolated from diseased specimens collected in various northern tobacco growing districts. As far as symptoms are concerned the disease in question is undoubtedly identical with that first described by Wolf and Foster in North Carolina. Isolations from North Carolina material have been included in our studies. There are at least two morphological characters, and certain cultural characters, in which our organism differs from the original description. It is believed that the morphological differences may be of sufficient interest to warrant mention here. For comparison the data may be summarized as follows:

Source of data	Extreme dimensions in microns		Average size in microns		Number of flagella
	Width	Length	Width	Length	
Original description, Wolf and Foster, 1917	0.9-1.5	2.4-5.0	1.2	3.3	1
Strains collected in Connecticut, Kentucky and other states	0.5-0.75	1.4-2.8	0.6	1.7	3-6

The wildfire disease has caused concern in several tobacco growing states where it has recently been introduced. Since at least two other bacterial leaf spot diseases of tobacco have been described in the United States, the importance, from a diagnostic standpoint, of checking the morphology of the wildfire organism is evident.

*Experimental evidence relating to the nature of the mosaic virus.* JAMES JOHNSON.

Chambers in which plants can be grown under controlled temperature and humidity conditions have been constructed. It has been found that the optimal temperature for the mosaic disease of tobacco lies close to 28-30° C. The maximal temperature for its expression is approximately 37° C., that is, at this temperature inoculated plants fail to develop symptoms, and leaves showing mosaic symptoms gradually "recover." Similar results may follow from low temperature exposure. Quantitative determinations of enzymes, said to cause mosaic, indicate that enzymes are not correspondingly reduced by exposure of plants to the higher or lower temperatures; in fact, it is probable that the optimal temperature for their activity lies close to 37° C. It seems, therefore, that these results furnish evidence against the enzymatic theory of mosaic while at the same time they favor parasitic hypothesis, since the temperature curve for the development of mosaic corresponds closely with that of the development of many of the plant pathogens.

*Non-parasitic leaf spots of tobacco.* JAMES JOHNSON.

The tobacco leaf is subject to a great variety of spot diseases, commonly called "rust." The list of these shown to be of parasitic origin is growing, but many are non-parasitic in nature, as has been shown by repeated attempts at culturing from such spots. Many opinions as to the cause of these spots are to be found, principally in semi-scientific literature, and experiments are being conducted to test various plausible theories. These spots can not all be assigned to the same cause, and the symptoms from any one cause may vary considerably. An attempt is being made however to classify these spots as to symptoms and cause. Tentatively they are found to fall into four main groups, namely, those due to: 1. an inherent physiological predisposition to spotting, 2. to unbalanced nutrition, 3. to absorption of toxic agents, and, 4. to toxic agents applied externally. These groups may be illustrated by the following cases: Certain varieties, especially Sumatra and Connecticut Broadleaf, commonly show spots when other varieties do not. A strain originating from a cross between two varieties not predisposed to spotting became extremely spotted. Shortage of phosphorus seems to be a predisposing factor. Certain soils rendered toxic by sterilization may produce marked spotting. Spotting also commonly results from spraying with insecticides. The prevailing environmental conditions seemingly affect the expression in all cases.

*The stem and bulb infesting nematode in America.* G. H. GODFREY.

During the past season the stem and bulb-infesting nematode, *Tylenchus dipsaci* (T.

*devastatrix*) has been found to occur in America on red clover, alfalfa, strawberry, and daffodils. On the first three hosts it is doing serious damage locally in Oregon and Idaho. Yields are reduced and attacked plants are killed prematurely. On red clover and alfalfa pronounced swellings occur on the above-ground parts of the plants. In the late fall or early spring these swellings are as a rule confined to stem bases in the crowns of plants. During the growing season the nematodes are carried up with the growing stems and typical swellings may occur a foot or more above ground. On the strawberry, swellings or galls, sometimes accompanied by abnormal red coloration, occur on leaves, leaf-petioles, stolons, flowers, and flower pedicels. On daffodils the disease was found in a bulb garden in Chicago, attacking the leaves and causing small, yellowish, slightly swollen spots. In Europe the disease is serious on both daffodils and hyacinths, attacking the leaves first and later penetrating to the bulbs. In severe cases the plants become dwarfed and distorted. The disease should be watched for generally on all susceptible plants, and any new occurrence promptly reported.

*Fusarium rot of gladiolus.* L. M. MASSEY.

One of the three diseases of gladiolus most commonly met with is one to which the name "Fusarium-rot" has been given. The corms become infected in the field, and the rot advances in storage. Lesions on the corms are slightly sunken, more or less circular in outline, have definite margins, and frequently have definite and conspicuous concentric markings or zones. The color of the lesions varies somewhat with that of the corms, but is frequently hazel, bay, burnt sienna, Sanford's brown, or mahogany red, (Ridgway). A *Fusarium* has been consistently isolated from typical lesions. Inoculations both in the field and under glass have established the pathogenicity of the fungus. Assistance in the determination of the fungus was given by Dr. C. D. Sherbakoff, who concurred with the writer in his decision that the fungus agrees closely with *F. oxysporum* Schlecht. Certain minor morphological differences and the results of inoculations on several hosts indicate that the fungus should be given at least a new varietal name.

*Soil temperatures obtained under a steam pan.* N. REX HUNT AND F. G. O'DONNELL.

Soil temperatures under the steam pan were obtained by the use of electrical thermocouples buried at different levels in the soil under the pan. The initial temperatures were taken and steam turned on, temperatures being recorded every five minutes until the soil at all depths had begun to cool. Temperatures varied somewhat with the soil type and condition and with the condition of the steam (whether "wet" or "dry"). Variations in steam pressure had a marked effect on the rapidity of penetration. Steaming for seventy five minutes raised the soil temperature to near the boiling point for a depth of seven inches or more, using a 6 x 9 ft. steam pan, with steam at 90 lbs. pressure, from a  $\frac{3}{4}$  inch pipe. The pressure gauge was read before turning the steam on, as the pressure was lost as soon as the steam entered the pan. With forty minutes of steaming the following temperatures were obtained: At one inch, 98° C. in 45 minutes after steam was turned on; at five inches, 50° in 80 minutes; at eight inches, 37° in 125 minutes. The pan was removed thirty minutes after steam was shut off. The rise and fall of the soil temperature was somewhat irregular as shown by curves based on the data obtained.

*Printing plate cultures.* F. G. O'DONNELL.

The direct printing of plate cultures, while somewhat generally known is very little used. *Method:* Plates are placed on sheets of photographic or blue print paper and ex-

posed without removing the plate covers. Paper is developed as usual. A hard glossy paper gives the best results. Length of exposure must be determined by trial. Prints can be made at less than two cents apiece for materials. To facilitate printing of large numbers of plates an inexpensive printer was designed.

*The dissemination of plant diseases by seed.* C. R. ORTON.

In the profitable production of plant crops there are four factors of paramount importance to be considered. These are 1. the soil, together with the maintenance of its fertility; 2. the seed; 3. the protection of the growing crop against diseases, including insects; and 4. meteorology. The first three are subject largely to manipulation, the last is beyond control to any great extent. Of the three factors controllable by human agencies, that of the seed is of importance from five standpoints, viz. 1. inherent resistance to disease, 2. freedom from disease, 3. vitality, 4. quality, 5. productiveness and adaptability. Up to the present the development of improved seeds has been almost wholly along the last three lines. The question of resistance to disease has been attacked by several workers, with noteworthy results; that of freedom from disease has been agitated intermittently by various agencies and some progress made, but the national and international importance of this problem has apparently not been realized by those most concerned. At the present time the evidence is conclusive that many of the diseases of crop plants are disseminated upon or within the seed to a greater or less extent. It is undoubtedly this fact which accounts for the present wide distribution of many important plant pathogens. The importance of this situation should be realized by the public, as well as commercial interests and scientific workers. A full discussion of this problem and the methods of organization for its attack is desired.

*Third progress report on apple scab and its control in Wisconsin.* G. W. KEITT.

The studies previously reported have been continued and a series of dusting experiments added. The early spring and summer were so dry that scab development was insufficient to furnish an adequate test of the efficiency of the various spray and dust programs. The first ascospore discharge was observed at Sturgeon Bay on May 13 and the last on June 16. No heavy or protracted discharges occurred. Inoculations on leaves and fruit in the experimental orchards during the last two years have shown incubation periods of from 14 to 18 days. Orchard observations in 1916 and subsequently and infection experiments in the last two years have shown that leaves and fruit of the varieties studied are much more susceptible to scab when young than in later stages of development. The upper surfaces of leaves ordinarily became highly resistant before they were fully expanded, while the lower surfaces of mature leaves might develop a diffuse, sooty type of infection after a much prolonged incubation period. These phenomena appear to be significant in relation to the nature of disease resistance.

*Susceptibility of apple root-stocks to black root rot.* F. D. FROMME.

Inoculation of apple trees on seedling roots with *Xylaria* sp. (*X. digitata*?), the species which commonly causes black root rot of apple in Virginia, produced infection and death of three-fourths of the trees within a period of three years. Similar inoculation of trees on Northern Spy roots produced infection of only one-fifth of the trees. One-third of these were only slightly infected. The others died within three years. Similar resistance was shown by other Northern Spy rooted trees which were set in orchards as re-

plants following trees killed by root rot. The Northern Spy root appears to be markedly superior in resistance to the seedling root stocks used by nurserymen.

*Origin of apple-blotch cankers.* MAX W. GARDNER.

Observations in Indiana on blotch (*Phyllosticta solitaria*) on Northwestern Greening have shown that a very high percentage of the twig cankers occur at leaf scars. Basal petiole lesions are very abundant on the leaves of the lower limbs. Many cases have been observed in which the petiole lesion had actually crossed over to the twig in the fall, but most of the cankers do not appear until the second season. Careful observations between September, 1920, and September, 1921, have shown that most of the cankers on the 1920 twigs appeared during April and May of 1921. Cultural tests, in which the fungus was isolated from petiole segments and leaf scars well below the lower margin of a petiole lesion, indicates that the mycelium may grow down within the petiole and cross the abscess layer before the leaf falls. Another type of twig lesion seems to result from bud scale infection. Direct infection of suckers and water sprouts between the leaf scars is of common occurrence. The standard blotch-spraying program gives an almost perfect control of petiole infection and apparently of twig infection.

*Studies on the infection and control of crown gall on apple grafts.* I. E. MELHUS AND T. J. MANEY.

Infection and possible control of crown gall on apple grafts has been studied for the past five years at the Iowa State Experiment Station. It has been found that infection of apple grafts is readily accomplished by dipping the grafts, just before planting, in a viable bouillon culture of *Bacterium tumefaciens*. The majority of the galls occur at the union. The stock is less liable to become infected than the scion. Grafts are equally susceptible to the organisms whether the callus is normal, excessive, or slight. Most of the infection takes place the first year during the formation of the callus at the union. Well-made and poorly-made grafts showed little difference in the amount of infection. Using an unusually large, heavy string wrap over the union leads to girdling and excessive callusing of the trees, which seem to facilitate crown-gall infection. Cloth applied over the union as a wrapper, either with or without string, decreases the amount of crown gall. Scion wood cut from trees infected with crown gall at the union, did not show any increased amount of infection. Hairy root seedlings when used as stocks did not transmit hairy root to the scion, but the stock portion of the graft usually remained infected. Surface disinfection with soluble fungicides is injurious to the grafts. Fungicides which go into solution slowly, such as lead arsenate and Bordeaux mixture, have a much less injurious effect on the callusing process. A strong Bordeaux mixture, 25-25-50, decreases the amount of crown gall, but also decreases the stand. This dilution has a marked preserving action on the string wrapper, which tends to aid girdling. A resin sticker added to Bordeaux mixture increases its toxic action and reduces the stand. The addition of lead arsenate or soaps to Bordeaux mixture does not increase its toxic actions on the grafts, but rather increases its adhesiveness and its fungicidal efficiency. More dilute Bordeaux mixtures did not reduce the stand and proved nearly as beneficial in reducing crown gall as the stronger mixtures. The use of Bordeaux mixture, 8-8-50, with or without lead arsenate, reduced the percentage of crown gall about 66 per cent over the checks, and nearly 50 per cent over the mean per cent of crown gall in all the checks in the Wealthy variety.

*Studies of crown gall.* A. J. RIKER.

Studies of crown gall on tomato and raspberry have been made at Madison, Wis.



during the last two years with an organism isolated from black raspberry, which conforms in most respects with the description of *Bacterium tumefaciens* Smith and Town. No standard varieties of red or black raspberries were found to be strikingly resistant to this disease. No evidence obtained indicated that infection had an immunizing effect. Infection in tomato was secured only through wounds and at temperatures below 30° C. The organism was positively chemotactic to juices of crushed raspberry or tomato tissue. It is living after a year in sterile soil. In saturated sterile soil it migrated at the rate of about a centimeter a day. An effort was made to locate the organism in the tissues. The evidence secured from microscopic study of the tissues at different stages of gall formation and from the development of the original and secondary galls, indicated that it was living between the cells of the host. Its intercellular position and its scarcity apparently contribute to the difficulty of demonstrating it in the tissues. Under certain conditions the organism was found to travel through the vascular bundles. This resembles a method of distribution of cancer in animals.

*Studies on Plant Cancers, IV—The effect of inoculating various quantities of different dilutions of Bacterium tumefaciens into the tobacco plant.* MICHAEL LEVINE.

Tobacco plants of uniform age and size and growing under uniform conditions of soil and light were inoculated with *Bacterium tumefaciens*. In these experiments I have not counted the organisms, but have used suspensions in small quantities, varying from the ordinary agar culture emulsions to dilutions equal to 1:100, to determine the effect of these varying quantities of bacteria on this host. Of 350 plants so tested, no marked difference could be detected in the size of the crown galls resulting. The inoculation of a drop of the weakest suspension of the crown gall organisms in a growing region often incited crown gall development much larger than a suspension forty times more concentrated. A comparison of the reaction of different parts of the plant to suspensions of equal dilutions was made. It was found that decapitated stems, inoculated with *Bacterium tumefaciens* into the cut end of the stem invariably produced the largest crown galls. Stems uninjured produced crown galls next in size, while the crown galls in the midveins of the leaves were smaller. A test of the relative virulence of three different ages of cultures was made. The cultures used were subcultures of *Bacterium tumefaciens* of the hop strain which had been grown on bean agar for the past three years and transferred to fresh media at intervals of about a month. It was found that cultures two days old were no more effective in producing crown galls in tobacco than were cultures seven days old and three weeks old. The sizes of the crown galls resulting from apparently equal dilutions of these cultures were approximately the same as those from unequal dilutions. It is concluded that the number of cells of *Bacterium tumefaciens* inoculated into the tobacco is not significant in determining the size of the crown galls produced. A smaller number of bacteria favorably lodged in tissue capable of response will produce a crown gall equal in size to that produced by a larger number. The size of the crown gall is rather dependent upon the region of inoculation and the vitality of the host than on the number of bacteria causing the infection.

*A new peach wilt disease.* C. M. HAENSELER.

During the summer of 1921 a new peach-wilt disease affecting from five to twenty per cent. of the trees was observed in several two to four-year-old orchards in Camden and Burlington counties, New Jersey. The disease started when the new growth was in its most rapid development, became most severe during a June drought, and ceased further development after August. Diseased portions of a tree show a shedding of the older leaves, wilting of the growing tips, and subsequent death of the twigs. Generally one or more of the main branches die, the rest of the tree remaining apparently

normal. Darkening of the wood portion of all affected parts, new wood as well as one to three-year-old wood, is characteristic. This wood discoloration can generally be traced from the diseased shoot down the trunk to below the ground level, where infection apparently takes place. Microscopic examination of new and old diseased wood shows a copious fungus growth in the wood vessels. Tissue cultures from over thirty twigs taken from different orchards gave a species of *Verticillium* in approximately 75 per cent. of the cases. This *Verticillium* was compared with *V. albo-atrum* from okra and egg plant but no marked morphological or cultural differences could be noted. Inoculation experiments are in progress. A possible relation between this *Verticillium* wilt of peach and winter injury is suggested.

*Relative susceptibility of citrus plants to Cladosporium citri Massee.* GEORGE L. PELTIER AND WM. J. FREDERICH.

In connection with our citrus canker investigations in Alabama, an opportunity was afforded to note the relative susceptibility of Rutaceae plants to scab. With the exception of the trifoliolate orange, all relatives tested are non-susceptible, and scab appears to be strictly limited to the citrus fruits and their hybrids. The pointed leaf form of "Cabayao," mandarins, calamondin, and kanzu oranges, all resistant to canker, are susceptible to scab. Grape fruits vary in susceptibility, while all lemons are susceptible. No scab has been observed on plants of the sweet orange group. Trifoliolate and citrange hybrids vary from slightly to very susceptible. The citrangequat (citrange x kumquat) is extremely susceptible to scab, although kumquat is outside the range of scab susceptibility, while the orange of the citrange cross is non-susceptible. On the other hand, this hybrid is the most promising canker-resistant plant so far found. The orangequat (sweet orange x kumquat, both non-susceptible), is quite susceptible to scab. The bigaraldin (sour orange x calamondin, both susceptible to scab) has remained non-susceptible. Judging from the results obtained, scab susceptibility is not as clear cut as that observed for canker, and appears to be influenced by the reaction of the host plant to environmental conditions essential for scab infection and subsequent development of the disease.

*Weather and its relation to citrus scab epidemics in Alabama.* GEORGE L. PELTIER AND WM. J. FREDERICH.

Under Alabama conditions, temperatures for optimum infection usually prevail during April and May. Sufficient moisture is generally at hand during this interval for successful infection to take place. The most important and variable factor is the development of the first spring growth. Any environmental factor or factors inducing a slight spring growth and rapid maturation favors escape of scab, while any environmental factor or factors inducing a large amount of spring growth and slow maturation favors scab susceptibility. The conditions essential for an epiphytotic are a late season, sufficient moisture, and the development of spring growth at the time optimum temperatures for infection prevail. An early season is favorable to scab escape, in that the first spring growth is about complete when optimum conditions for infection are at hand. Under Alabama conditions, a light or bad scab year can be predicted by the monthly mean temperature prevailing during March; a monthly mean temperature below normal is an indication of a bad scab year; while a normal temperature (59° F.), or above, is indicative of a light infection.

*A preliminary report on the control of raspberry anthracnose.* LEON K. JONES.

In 1920 and 1921 experiments on the control of raspberry anthracnose were conducted at Madison, Wisconsin. Lime-sulphur, alone and in combination with gelatin, glue, and saponin, respectively, and Bordeaux mixture, alone and in combination

with gelatin, glue, milk, and caseinlime, respectively, were tested comparatively on Cumberland black raspberries. Two applications were made, 1. after the first two or three leaves had unfolded, and 2. about one week prior to the opening of any blossom buds. Each of the fungicides, alone and in combination with its various adhesives, was used in the following programs: 1. Both applications, 2. first treatment only, and 3, second treatment only. Lime-sulphur, 1-10, and Bordeaux mixture, 6-6-50, were used in the first applications, and lime-sulphur, 1-40, and Bordeaux mixture, 3-3-50, in the second. In both seasons the disease was satisfactorily controlled by spraying, lime-sulphur giving better results than Bordeaux. With lime-sulphur, glue and gelatin gave the best results as adhesives; with Bordeaux mixtures, gelatin and caseinlime. The two-spray program gave satisfactory control each season. In 1921 the first application alone, with lime-sulphur and gelatin, controlled the disease satisfactorily on plants which had been well sprayed the previous year. The second application alone failed to control the disease in any case. On unsprayed plants the disease developed abundantly in both seasons.

*Leaf curl and mosaic of the cultivated red raspberry.* W. H. RANKIN, J. F. HOCKEY AND J. B. MCCURRY.

These diseases, previously confused under the name "yellows", are distinct and easily separable. Both diseases are systemic and cause dwarfing. In leaf curl the leaflets are very dark green, and the midrib and main lateral veins arch downward, causing a curling of the entire margin of the leaf. The tissue arches between the veins and causes a puckering along the veins. In mosaic, the leaflets on new growth in spring show large green blisters, with yellow-green tissue between. In summer and autumn the mottling is much finer and gives the leaf a uniform yellowish, speckled appearance. On fruiting canes the leaves are either coarsely or finely mottled and reach only one-half normal size. In the Niagara district of Ontario leaf curl is enphytotic in Cuthbert, affecting from five to ten per cent. of the stand. Mosaic is epiphytotic in Cuthbert and Marlboro, to the extent of an average infection of twenty to thirty per cent. Only one disseminating agent, *Aphis rubiphila*, is suspected for both diseases. Leaf curl transmission by this agent has been proved. No causal organism for either disease has been found. Control of mosaic is anticipated by roguing in August, thus preventing aphid eggs overwintering on diseased wood. Leaf curl has been reduced by a single roguing in July for two years from 4.4 per cent. in 1919 to 1.9 per cent. in 1920 and 0.0 per cent. in 1921. Roguing for leaf curl as soon as the bushes leaf out will give commercial control. The bushes in the case of both diseases must be dug carefully to get the entire root and removed immediately to a distance to prevent the migration of the aphids to healthy bushes.

*Records for four years on the needle blight of Pinus strobus.* J. H. FAULL.

"Needle blight" of *Pinus strobus* has been reported by the Forest Service and by lumbermen many times from 1905 onward. Investigations were begun in 1918. The disease manifests itself as a reddening of the new needles and has been so abundant that certain pine areas have assumed an autumnal coloration in midsummer. The trouble has been variously ascribed to winter injury, late frosts, insects, fungi, etc., and has been confused with winter browning and sulfur fumes injury. It has been discovered that it begins with a killing of the roots, apparently due to a combination of soil peculiarities and drought conditions, hence the root system is not able to supply the sudden demand for water made by the new foliage. Repeated blighting results in the death

of affected trees. Hundreds of trees were examined in 1918 and tagged with serially numbered metal disks. Out of 275 healthy trees 2 have since developed blight and under exactly known conditions. Out of 147 trees 6 inches in diameter or less 7 per cent. have died. Out of 211 trees over 6 inches in diameter 23.7 per cent. have died. The results so far show that young stands for the most part recover, but that mature stands are seriously injured.

*Chemical injuries to white pines.* WALTER H. SNELL AND N. O. HOWARD.

During the summer of 1921 two non-parasitic troubles of the white pine came under observation. In one case in Massachusetts a lot of 25 to 30 acres of white pines, which appeared from a distance to be totally dead, were found to be still alive, but only the basal portions of the needles were living. Gases from the chimney of a brick kiln about  $\frac{1}{4}$  mile north were suspected as the cause of the damage. A checking of the weather records, with the dates of burning of the kiln, substantiated the suspicions that such gases (probably  $SO_2$ ) caused the trouble. Another case of the death of pine trees was along the roadside in New Hampshire. It was found that barrels of calcium chloride for application to the road had been stored under these trees and the salt which seeped into the soil had killed the pines and partially defoliated the elms and birches nearby.

*Hypoxylon poplar canker.* ALFRED H. W. POVAH.

This disease, caused by *Hypoxylon pruinaum* (Klotsch) Cke., has been found in Essex and Oswego Counties, New York, in Michigan and in Maine. The disease is a trunk canker which kills the bark. Often the tree is girdled, which results in the death of the whole upper part. Usually the dead top is sooner or later broken off by the wind. A survey of a sample plot in Essex County, N. Y., showed 37 per cent. of *Populus tremuloides* infected and 27 per cent. of them killed by this disease. A diagnostic feature of the disease is the blackening of the sapwood. When the bark is peeled from a canker, the discoloration shows as points, extending vertically, on which are found fans of white mycelium.

*The effect of heat upon the mycelium of certain structural timber destroying fungi within wood.* WALTER H. SNELL.

Spruce blocks  $\frac{3}{4}$  inch in cross section thoroughly grown through with *Lenzites sepiaria*, *L. trabea*, *Trametes serialis*, *T. carnea*, and *Lentinus lepideus* were submitted to various degrees of heat for varying periods in wet and dry atmospheres to determine the thermal death point of the mycelium within wood. None of the fungi could survive 55° C. moist heat for 12 hours and only one (*Lenzites trabea*) withstood 3 days at 44° C. moist heat. At 60° C. dry heat, the time necessary to kill the mycelium was 5 to 12 days, varying with the species. At 90° C. only *L. trabea* could survive 24 hours, and not until 105° C. dry heat was reached would 12 hours exposure kill the mycelium of all the fungi. It is concluded that heating structures affected with decay to 47–48° C. by means of the heating systems, as has been suggested, would not kill the fungi even in moist cotton weave sheds, although the drying effect would be beneficial in certain types of structures. The application of these results to the effect of kiln drying upon structural timber decay is pointed out.

*The occurrence and development of pathological resin canals in the Coniferae.* ARTHUR S. RHODES.

The writer has made an extensive study of pathological resin canal formation in a large number of the Coniferae, with regard to the various causes stimulating their development, the manner and extent of their occurrence as a result of numerous diverse

types of wounding, and the pathological anatomy of such resin canal formation and its biological and phylogenetic significance. The conditions stimulating the formation of pathological resin canals may be classified as follows: 1. Injuries to the cambium resulting from all kinds of mechanical wounding, including those occasioned by animals and insects; 2. attacks by various parasitic fungi and mistletoes; and 3. abnormal physiological conditions of growth and nutrition, in which the direct influence of wounding or of attacks by parasitic plants is lacking, which produce a pathological condition of the plant. While usually of purely local extent in most mechanical injuries, pathological resin canals may form continuous rows, extending entirely around the growth ring and for many feet vertically, as often occurs in lightning injury and severe cases of sap-sucker injury. By reason of its anatomical structure, a zone of pathological resin canals constitutes a decided line of weakness. The use of small pieces of wood containing such formations should therefore be discriminated against for purposes requiring great strength, as in aeroplane parts, since they are very likely to fail under such stresses as would invite cleavage or shearing with the grain. Pathological resin canals are now known to occur in all the genera of the Abietineae and in the genus *Sequoia* of the Taxodineae, and occur in as many species of these genera as have been investigated in this respect. Neither their presence nor absence nor their position within any part of the growth ring can be of diagnostic value. Their formation in the conifers is paralleled by the formation of analogous structures in a number of dicotyledonous woods.

*Helminthosporium heveae* Petch, in Sumatra. CARL D. LA RUE.

*Helminthosporium heveae* was described by Petch on *Hevea brasiliensis*, the Para rubber tree, more than fifteen years ago. Bancroft in 1911 stated that there was no record of its occurrence in the Federated Malay States. Butler in 1918 says the disease occurs in Malaya, Ceylon, South India, and Java. Both Petch and Butler state that the fungus is confined to young rubber trees. This is usually true in Sumatra, but in 1919 the writer found it on old trees on numerous estates. In some cases the leaves were riddled with spots and the injury must have been very considerable. The disease does not cause defoliation of the affected trees, and where defoliation occurs it is usually found to be due to a simultaneous attack of mites. The fungus attacks the leaves and occasionally the bark of young twigs. Infection occurs just as the young leaves unfold, and the old leaves, either fallen or still hanging on the trees, are probably the source of the infecting spores. The fungus is easily grown in culture but fruits slowly. The Sumatran forms agree with Petch's description, except that the spores are rather small.

*Pulp storage in water.* R. J. BLAIR.

Ground wood pulp, which is manufactured by the simple processes of holding sticks of wood against a revolving grind-stone, often seriously deteriorates in storage through the action of molds and wood-destroying fungi. Invasion by such organisms is rendered easy, as the material is always stored in a moist condition. It is an accepted fact that wood immersed in water is immune to fungus attack. An experiment was carried out using several kinds of commercial pulps in order to test the preservative value of water upon sheets of pulp immersed in it. After an interval of seventeen months the pulp was examined and tested for freeness. It was then made into small sheets of paper, which were tested for bursting strength and for tensile tear. The pulp stored in water came through the test in much better condition than that which was piled on a shed where it was given an opportunity to dry out.

# PHYTOPATHOLOGY

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## CERCOSPORA LEAF SPOT OF EGGPLANT

COLIN G. WELLES

WITH TWO FIGURES IN THE TEXT

A serious spotting of the leaves of eggplant (*Solanum melongena* Linn.) has been observed recently in the Experiment Station, at the College of Agriculture, Los Baños, Laguna Province, Philippine Islands. Leaf spotting of eggplant is quite commonly caused by the fungus, *Phyllosticta hortorum* Speg. but the leaf spot about to be discussed is not similar to it in any respect. This paper presents the results of studies made on the disease with special emphasis on the causal organism and control.

### THE DISEASE

*Symptoms.* The spots occur in abundance on the leaves, the old leaves being most seriously attacked. The young lesions appear as chlorotic spots, irregular in shape and frequently fusing, giving the leaf a yellow, mottled appearance. The irregular shape is due to the limiting of the lesions by the veins of the leaf. The young lesions develop on leaves of all ages but chiefly on the lower, old leaves. They first appear on the upper surface of the leaf, later extending through the tissues and causing a spot on the under surface as well. When they become older the central portions of the lesions dry out and turn grayish-brown or dirt color due to the death of the cells, and exhibit concentric rings. With age these dry areas may become 6 to 8 mm. in the largest dimension. The dry portions develop first at or near the margins of leaves and spread inward frequently as much as 20 mm. These lesions are always surrounded by a band of yellow tissue. In very advanced stages of spotting, the dead tissues fall out leaving a shot-hole condition.

The fruit of many plants has been carefully examined but no lesions have been found which can be traced to the *Cercospora*.

No disease of eggplant has been described as far as can be ascertained, which approaches the appearance caused by this fungus more closely than the leaf spot due to *Phyllosticta hortorum*. However, the lesions of this disease are larger in size and lighter in color than the *Cercospora* leaf spot under discussion. Furthermore, the pycnidia of the *Phyllosticta* are very pronounced whereas the *Cercospora* lesion is evenly colored and unbroken by any dark sporiferous bodies.

*Varietal attack.* The *Cercospora* is confined to eggplant so far as is now known. Within this host species, however, observations point to varieties showing differences in susceptibility to this parasite. The native Philippine eggplant which has long, black fruit, turning yellow at maturity, is very seriously parasitized while a Siamese variety having small, round, yellow fruit is but slightly affected. The native variety was seriously attacked from the end of the dry season but the Siamese variety showed no signs of disease until nearly four weeks later.

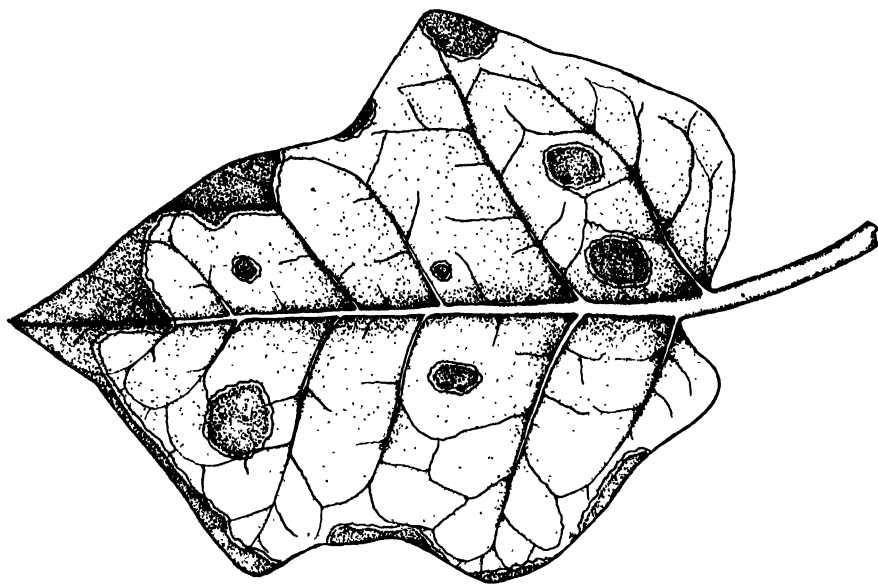


Fig. 1. Leaf spot of eggplant caused by *Cercospora melongenae*.

Drawing by C. C. Nacion.

*Economic importance.* Plants grown in the Experiment Station are severely attacked by the *Cercospora*. On fully matured leaves the photosynthetic area of the leaf is reduced more than 75 per cent. On young leaves which are still developing, the lesions are confined to the margins with a reduction of photosynthetic tissue often reaching 30 per cent. All plants in the field are affected by the fungus with from 50 to 100 per cent. of the leaves attacked.

*Distribution.* The disease has been observed only on plants in the Experiment Station. A survey of the market gardens of the Philippine Islands will be necessary before the extent and seriousness of the disease in other places can be ascertained.

#### THE CAUSAL ORGANISM

*Morphology.* The organism which causes the leaf spot of eggplant belongs to the genus *Cercospora*. The fruiting bodies are borne in the grayish-brown center of the lesions, mainly on the lower surface of the leaf. The conidiophores are of the common *Cercospora* type, arising in bunches from the stomatal openings. They are rather short, simple, light brown, and erect. They measure from 30 to 60 microns long and 4 to 6 microns wide and are 2 to 4 septate. The conidia are linear, slightly larger at the basal end, tapering gradually to a blunt point and hyaline. The average conidium measures 74.85 by 7.14 microns, the extremes being 38 to 119 microns long and 4.7 to 8.3 microns wide. They are 3 to 12 septate.

*Cultural characters.* Germination took place in 12 hours in our trials. The conidia germinated by slender germ tubes, one, two, or three generally arising from each conidium from as many cells. The fungus may be isolated by the single spore method in potato agar or by plating diseased tissue directly on corn meal. The mycelium development is rapid. On potato-glucose agar the mycelium when young is white and fluffy and grows close to the medium, later becoming a very thick, closely matted mass with a grayish-green center encircled by a band of white mycelium. The cultures have been kept for five weeks but no fruiting structures have developed.

Since the organism is apparently a new species the following name and description is submitted:

***Cercospora melongenae* n. sp.** Spots amphigenous, indefinite, grayish-brown, 6 to 8 mm. in largest dimension, concentric rings. Conidiophores mostly hypophyllous, short, light brown, fasciculate, 30–60 x 4–6 microns, 2–4 septate. Conidia filiform-obclavate, straight or curved, 38–119 x 4–8 microns, averaging about 75 x 7 microns, 3–12 septate, hyaline.

Found on eggplant, *Solanum melongena* Linn., in Los Baños, Laguna Province, Philippine Islands.

#### CONTROL

Several experiments have shown that spraying with Bordeaux mixture every two weeks has sufficed to hold in check the development of the leaf spotting of the eggplant. However, comparison of such sprayed plants with the unsprayed controls has shown no important difference so far as relates to the setting or development of the fruits. On the other hand, the spraying does increase the longevity of the leaves and



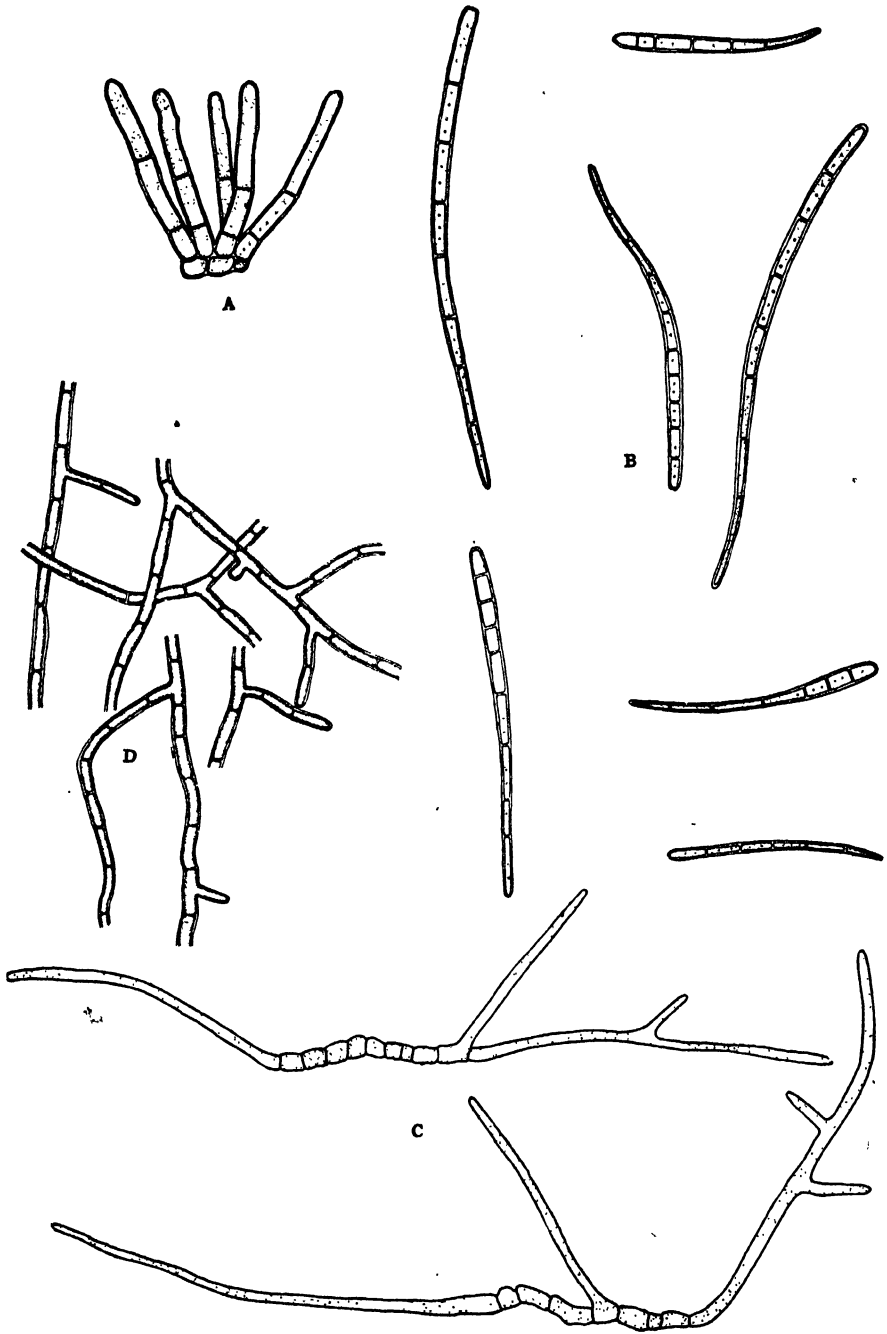


Fig. 2. *Cercospora melongenae*: A, group of conidiophores. B, conidia. C, germinating conidia from potato-glucose agar plates. D, mycelium from pure culture, on potato-glucose agar. Camera lucida drawing by E. Roldan and C. C. Nacion.  $\times 460$

the general vitality of the plants. From these results it seems that spraying for such control purposes is not commercially justified for mature plants unless the disease threatens to be so unusually severe as to cause serious defoliation. Should this occur, especially with young foliage or young plants, spraying may be relied upon as a control measure.

#### SUMMARY

1. A previously unreported *Cercospora* leaf spot of eggplant has been found in the Experiment Station for the College of Agriculture, Los Baños, Philippine Islands.

2. The lesions look somewhat similar to those caused by *Phyllosticta hortorum* Speg. The general appearance of the lesion is the only point of likeness however.

3. It attacks all local varieties but is most severe on the native Philippine eggplant. It is less parasitic on a Siamese variety with round, yellow fruit.

4. It may reduce photosynthetic tissue as much as 75 per cent.

5. The causal organism is described as a new species under the name *Cercospora melongenae*.

6. The leaf spotting is easily reduced by spraying with Bordeaux mixture. Since this has not materially increased the yield with mature plants it may not be commercially profitable unless the attack appears with unusual earliness or severity.

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# THE TAKE-ALL DISEASE OF CEREALS AND GRASSES

R. S. K I R B Y<sup>1</sup>

WITH PLATES II TO IV AND THREE FIGURES IN THE TEXT

The take-all disease of wheat was discovered for the first time in the United States in July 1920 (13). Since that time extensive investigations have been made at Cornell University and the results are presented in this paper.

The name take-all has been generally applied, in Australia, England, and other English-speaking countries, to a disease produced by *Ophiobolus cariceti* (B. & Br.) Sacc. While it has been applied to forms of foot rot which are due to other causes, it seems desirable to restrict the name to the disease caused by this species of *Ophiobolus*.

## GEOGRAPHICAL RANGE OF THE DISEASE

An extensive historical and bibliographic treatment of various foot rots of wheat has been published by Stevens (26). Since many writers have only recently associated *Ophiobolus* specifically with the foot rot which it causes, many statements in the older literature are of little value in determining the host range of the fungus. The causal organism has been known since 1861 (1), when it was described from specimens collected at Batheaston, England, on *Aira caespitosa*. In 1875 it was described as occurring on *Agropyron sp.* and *Cynodon sp.* in Italy (24), and in 1878 it was noted on wheat in France (20). Take-all of wheat was found in England in 1884 (10), and in 1890 it was described in France (22). McAlpine (16) found the disease widespread on wheat in South Australia in 1904, and he states that it has been known since 1852.

Take-all has been observed in France, England, Holland, Belgium, Portugal, Italy (19), Australia (16), New Zealand (31), Japan (29), Denmark, Russia (7), and the United States (13).

<sup>1</sup>Suggestions and help were received from Doctor H. E. Thomas during the earlier part of these investigations.

As herein defined, it was first recorded in the United States by Kirby and Thomas (13). However, since that report was published, the disease has been found in Oregon (11), Arkansas (11), Indiana,<sup>1</sup> and additional localities in New York State (12).

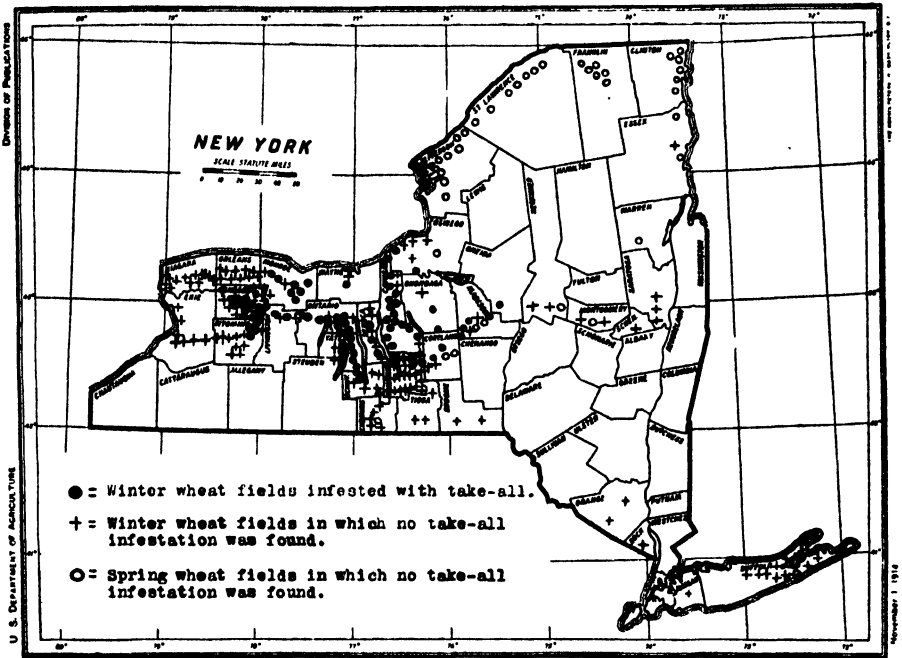


FIG. 1. LOCATION OF ALL CEREAL FIELDS SURVEYED IN 1921 WITH LOCATION OF TAKE-ALL INFESTED FIELDS.

A survey was made in 1921, in cooperation with the office of the Plant Disease Survey of the United States Department of Agriculture, to determine the distribution of take-all in New York State. The survey extended over thirty-six counties and included all sections where an appreciable amount of spring or winter wheat was grown. The location of all take-all infested fields, as well as all other winter and spring wheat fields surveyed, is shown in figure 1.

Plants affected with take-all were found in 78 of the 224 winter wheat fields surveyed. All of the 78 infected fields were located in sixteen counties in the west central part of New York. This infected area seems to have definite boundaries and closely coincides with the principal winter-wheat-producing area of the State. Within this area, fields representing more than 60 per cent of the total acreage surveyed were found to be infected.

<sup>1</sup>Reported to the writer in a letter from Professor H. S. Jackson, dated July 2, 1921.

An accurate determination of the amount of damage caused by take-all was impossible because many of the plants were killed in the seedling stage. However, some estimate of the resulting damage may be made from the fact that an average of 2 per cent of the plants in the 78 infected fields were killed by the take-all fungus. Therefore, since the yield of infected plants appeared to be about 1 per cent of that of normal plants, the loss in infected fields due to this disease can be placed conservatively at about 2 per cent.

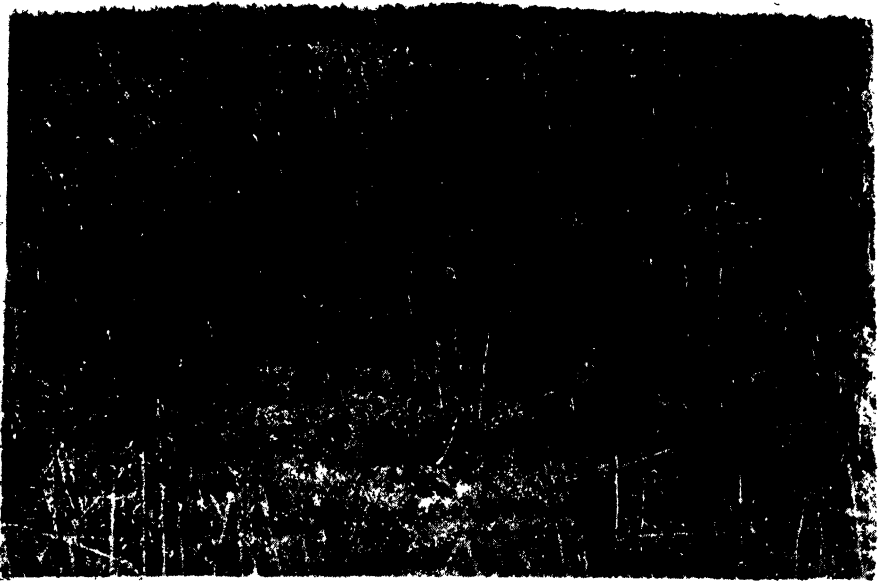


Fig. 2. A WINTER WHEAT FIELD AT SPENCERPORT, N. Y., AT HARVEST TIME SHOWING AN AREA IN WHICH PLANTS HAVE BEEN KILLED BY TAKE-ALL.

#### SYMPTOMS

The writer has not had the opportunity to study the disease through all its early stages in the field. The plants in the diseased areas at flowering time are stunted and bleached yellowish in color. From one to many plants are found in the infected areas which may attain a diameter of 15 feet or more and are usually circular in shape. (Fig. 2.) The edges often overlap in badly infected fields, so that one may walk across such a field without leaving them. Sometimes the disease occurs for several feet along the drill rows. The individual plants in these areas are usually ashy white in color, and are dwarfed to a few inches in height. They are usually dead and there is seldom more than one head to a stool. This chlorotic condition of the entire plant suggests the

name "white heads," which is used by some writers to designate this disease. There is a dark brown or black zone at the base of the diseased culms which is due chiefly to dark fungous mycelium in the leaf sheaths and between the culm and the inner leaf sheath, where it occurs as a plate-like mass. This discoloration is confined to the first and second internodes and may extend from one-half to two inches above the ground.

When badly diseased plants are pulled up, the roots break at or very near the crown. (Pl. II, fig. A.) The larger roots near the crown have a woolly appearance, due to the formation of a large number of short fine rootlets. These roots appear to be thicker than the healthy ones and retain considerable amounts of soil which often gives them a characteristic clubbed appearance.

One of the characteristic symptoms is the marked reduction of the normal number of tillers, many of which die soon after their formation. In plate II, figure B is shown the contrast between a healthy plant and a typical diseased one.

There is no marked change in the symptoms from the time of flowering to harvest of the wheat. However, the diseased leaves and culms may become sooty on account of the growth of saprophytic species of *Cladosporium* and *Mucor*. (Pl. III, fig. A.) At harvest time this blackening of the heads is as characteristic as the white-head stage is at flowering time. After the death of diseased plants, or rarely before, the beaks of the perithecia of the pathogene may protrude through the outer leaf sheath of the plant. (Pl. III, fig. B.).

The symptoms of the one infected rye plant observed differed from those on wheat in that there was little or no stunting of the plant and only slight shriveling of the kernels. The mycelial plate was less pronounced, and fewer perithecia were developed.

The chlorosis and stunting was not conspicuous on *Agropyron repens* (L.) Beauv., but the base of the culms was darkened by hyphal strands which were scattered in and between the leaf sheaths instead of being aggregated in a definite mycelial plate. Perithecia, however, were generally not so numerous as on wheat.

COMPARISON OF THE SYMPTOMS OF TAKE-ALL CAUSED BY *OPHIOBOLUS*  
*CARICETI*, WITH "FOOT ROT" OR SO-CALLED "TAKE-ALL"  
DISCOVERED IN MADISON COUNTY, ILLINOIS

The disease called foot rot by Stevens (26) has several times been referred to as "take-all" (11) or so-called take-all (17). It has the following symptoms in common with the take-all as it occurred in New York in 1920 and 1921: the stunting and death of plants in rather definite spots in wheat fields; the blackening of the lower culm usually the first internode.

The take-all in New York differs from the Illinois "so-called take-all" in the following respects: between the leaf sheath and the culm of infected plants, there nearly always occurs a definite plate of brown mycelial strands (Pl. III, fig. C.) Typical *Ophiobolus* perithecia containing ascospores were found on plants in every infected field. From infected plants cultures of *Ophiobolus cariceti*, *Fusarium gramineum* Corda,<sup>1</sup> *F. avenaceum* (Fr.) Sacc.,<sup>1</sup> *F. diversisporum* Sherb.<sup>1</sup> and *Gibberella saubinetii* (Mont.) Sacc. were obtained, but *Helminthosporium* sp. never was isolated. *Helminthosporium* was often found associated with the plants in the later stages of the Illinois disease, which always lacked the perithecia and the typical plate mycelium of the take-all organism. The diseased plants in New York differ from those in Illinois also in that they exhibit hypoplastic instead of metaplastic tillering symptoms in the spring.

#### IDENTITY AND DESCRIPTION OF THE FUNGUS

In an earlier paper (8), the causal fungus of take-all was shown to be *Ophiobolus cariceti* which is identical with the take-all fungus in Australia, Japan, England, France, and Italy.

The take-all fungus has been grown in pure culture on the following agar media: potato, lima bean, cornmeal, oatmeal, prune, nutrient, Czapek, and 0.2 per cent, 2 per cent, and 5 per cent dextrose. The growth of the fungus on these various media was of three general types, examples of which are described.

#### GROWTH ON 0.2 PER CENT DEXTROSE AGAR

Growth 2.5 cm. in diameter after seven days; seldom reaching a diameter finally of more than 5 to 7 cm.

*Growth after seven Days:* The mycelium is submerged or wholly appressed. The individual fungous growth is hyaline, and is made up of feathery, rhizomorph-like strands resembling the feathery branching of a moss.

*Growth after thirty Days:* Appearance nearly the same as at end of seven days, except deep neutral gray<sup>2</sup> hyphal strands have appeared near the center. The rhizomorphic strands are appressed to the surface or submerged. The strands radiate toward the edge of the culture and do not unite nor run parallel. The cells of the rhizomorphs are from 16 to 45 $\mu$  long and from 4 to 5.5 $\mu$  in diameter, and contain from 10 to 25 guttulae. The cell walls are hyaline. Branches arise from near the tip of about every eighth to tenth cell, or about every 150 to 250 $\mu$ . The cells of the branches are very much more variable in size and shape than those of the main rhizoids, ranging from knob-like cells with a diameter of 5 $\mu$ , such as occur at the end of a few hyphae, to cylindrical cells resembling those of the main rhizomorphs. (Pl. IV, fig. A.)

<sup>1</sup>Determined by Doctor C. D. Sherbakoff.

<sup>2</sup>Ridgway color.

## GROWTH ON 2 PER CENT DEXTROSE AGAR

Growth 4 cm. in diameter at the end of seven days, covering the plate (9 cm.) in from four to five weeks

*Growth after seven Days:* The mycelium is hyaline, and is submerged or wholly appressed. It is much less characteristically rhizomorphic than in 0.2 per cent dextrose agar, but this character is evident. The individual strands are finer and more abundantly branched, and their margins are uneven and indistinct.

*Growth after thirty Days:* The surface of the fungous growth is of a deep neutral gray color. The mycelium is submerged or wholly appressed. The individual strands appear often distinctly frayed at the tip, forming a rather definite tuft which continues as lighter colored hyphae. Many ribbon-like rhizomorphic bands run irregularly over the surface of the medium. These bands are made up of from two to three (sometimes as many as six) strands of hyphae running parallel. They have fewer hyphal strands than are produced in cultures grown on potato agar. The cells of the individual strands are cylindric and have thick deep neutral gray walls. They are from 25 to 275 $\mu$  long and from 4 to 6.5 $\mu$  in diameter (averaging  $196 \times 5.5\mu$ ). The contents are hyaline and the cells contain from 0 to 40 guttulae. Branches arise alternately, usually one, rarely two, from the distal end of nearly every cell.

Another type of growth often occurs in which there are numerous individual strands, and occasional ribbon-like bands rarely consisting of more than two hyphal strands. The individual cells of this type are cylindric and often constricted at their septa; they have thick, deep neutral gray wall, hyaline contents and from 10 to 40 guttulae; they range from 15 to 200 $\mu$  in length (averaging  $30 \times 5.5\mu$ ). Branches arise from the tip of every eighth to fourteenth cell.

The branches terminate in hyaline hyphae whose individual cells are comparatively thin-walled, very variable in shape and size, ranging from 15 to 60 $\mu$  long and from 2 to 6 $\mu$  in diameter. The cell contents are guttulate. Anastomosing was observed. (Pl. IV, fig. D.)

## GROWTH ON POTATO AGAR 5.4 PH

Growth 7 cm. in diameter at the end of seven days, covering the plate (9 cm.) in from eight to nine days.

*Growth after seven Days:* The mycelium is hyaline, and is submerged or wholly appressed. The individual culture is abundantly and distinctly flexuous; its margin is even but not marked by a definite line, appearing finely frayed.

*Growth after thirty Days:* The mycelium assumes a more pronounced aerial habit, rising to a height of 2 mm. and growing on the sides of the petri dish. The aerial growth causes the surface of the colony to appear as if covered with a silvery bloom. The surface of the medium is thickly covered with tortuous, mixed ribbon-like rhizomorphic bands of hyphae. The surface of the colony ranges in color from a neutral to a deep neutral gray. The rhizomorphic bands are made up of from 2 to 8 strands of parallel hyphae. The cells of the individual strands are cylindric and have thick dark neutral gray walls; they are 75 to 260 $\mu$  long and from 4 to 6.5 $\mu$  in diameter (averaging  $184 \times 5\mu$ ); the contents are hyaline and sparingly guttulate. Nearly every cell in the strands is branched at its tip. The cells of the hyphal strands closely resemble those of the plate mycelium on the host. The branches terminate in thin-walled hyaline hyphae, the individual cells of which contain from 10 to 30 guttulae. The cells are very variable in shape and size, ranging from 15 to 50 $\mu$  long and from 2 to 6 $\mu$  in diameter. Anastomosing was observed. (Pl. IV, figs. C. and D.)



## PARASITISM OF THE TAKE-ALL FUNGUS

In an effort to prove the pathogenicity of the fungus, Delacroix (6), Mangin (18), and McAlpine (16) inoculated growing wheat with diseased straw or spore suspensions, obtaining typical symptoms of the disease in each case. The first inoculations with a pure culture of the fungus were made by Waters (30) in New Zealand on wheat seedlings grown from sterilized seed on sterilized soil in test tubes. Inoculated plants died in from twenty-eight to thirty-six days from the date of inoculation, while control plants were in good condition after fifty-eight days. Inoculated culms of wheat plants growing in pots did not become infected.

In the present investigation the relation of *O. cariceti* to take-all was determined by the established rules of proof. At harvest time the base of the culm of infected plants was overrun with species of *Fusaria*, molds and bacteria. Nevertheless, pure cultures were obtained by first immersing bits of the host having one or more perithecia, in a 1-2000 solution of mercuric chloride for from one to two minutes to kill as many bacteria as possible. The material was then placed under a binocular microscope where individual perithecia were separated from all bits of the host tissue. These were placed in a few drops of sterile water on a slide where they were crushed. This suspension of ascospores was usually diluted to the desired degree and then atomized with a pipette having a very small delivery orifice on the surface of the medium in a petri dish. By placing a large drop of the suspension on the surface of the medium, then spreading it thinly over the surface some ascospores could be removed without bacterial contamination.

Two-tenths of one per cent dextrose agar was used throughout the isolation work. This medium is transparent, retards bacterial growth, and the take-all fungus growing on it produces a characteristic growth distinguishable from that of contaminating fungi. Single spore cultures were then obtained by transferring to sterile media young colonies which were observed under the microscope to have arisen from one ascospore.

By the use of the preceding methods during November and December of 1920, seven isolations were obtained from single ascospores and seven more from groups of two or more ascospores. Since that time it has been possible to make isolations whenever ascospores were germinating. After obtaining the fungus in pure culture it was transferred and grown on the following kinds of media: potato, lima bean, cornmeal, oatmeal, prune, nutrient, Czapek, and 0.2 per cent, 2 per cent and 5 per cent dextrose. It was grown also on sterilized cornmeal, wheat, oats, rice, lima beans, bean pods, wheat stems, wheat heads,

barley stems, barley heads, and sweet clover stems. The growth of the fungus was carefully studied on each of these media and a careful search was made for spore forms. Typical perithecia developed in from three to four months on the sterilized sweet clover and wheat stems.

Small amounts of the inoculum consisting of fungous growth on wheat kernels from one or more of these isolation strains were placed in 156 out of 234 pots of wheat at planting time. Steam-sterilized soil was used in the greater number of the pots. The results for a greater part of this test are included under the varietal tests of the host-range determinations. At maturity the plants in all of the 156 inoculated pots showed typical symptoms of take-all, while no plant in any of the 78 check pots exhibited such symptoms. The symptoms were similar in all respects to those obtained in other inoculation experiments in which bits of diseased straw taken from field plants were used as a source of inoculum, and also to those observed in the field. *O. cariceti* was re-isolated several times from the discolored area of plants that had been inoculated with pure cultures, but it was never isolated from bits of the host plant taken above the discolored area at the base of the culm. The reisolated fungus agreed in every essential with the original isolations. Furthermore, the reisolated fungus, when used as an inoculum at planting time, caused typical symptoms of take-all on the growing wheat plants. There is no doubt, therefore, that the fungus *O. cariceti* is capable of causing typical take-all.

#### DETERMINATION OF HOST RANGE

The need of a complete knowledge of the host range becomes at once apparent in considering the means of eliminating the take-all fungus from infected areas. Wheat and barley have been reported as being affected more often than rye and oats (23, 2, 31), which, however, are not immune. Rice also has been reported as subject to this disease in Japan (29).

The following grasses have been listed as hosts for the take-all fungus: *Agropyron repens*, *A. scabrosum*, *Aira caespitosa*, *Bromus mollis*, *B. sterilis*, *Cynodon sp.*, *Hordeum murinum* (1, 24, 16, 2, 31).

All varieties of wheat are considered as susceptible by Pridham (21). The percentage of infected plants among the eighty-one varieties which he tested varied from 1 to 33.7.

Workers in general agree that red wheats are more resistant than white wheats. According to Pridham (21), the early varieties are much freer from the disease than are the late varieties, but this does not agree with the statements of Lindau (14) and of the Great Britain Board of Agriculture (10). No record of the infection of spring wheat has been found by the writer.

*Field Hosts.* Winter wheat and winter rye are the only cereals on which take-all was found in 1920 and 1921. Winter wheat appeared to be the principal host of the take-all fungus.

The following varieties of soft winter wheat were found affected: Number 6 Junior, Dawson Golden Chaff, Red Rock, Leap Prolific, and Velvet Chaff. Field observations indicate that these varieties are all fairly susceptible, but Number 6 Junior and Leap Prolific were more severely affected than Dawson Golden Chaff and Red Rock. A trace of take-all was found in one field of Turkey Red, but since only two fields of this variety were examined definite conclusions cannot be drawn.

In 1921 forty-four spring wheat fields, twenty-five fields of oats, and seven barley fields were examined carefully but no take-all was found.

Rye appears to be resistant except when growing under unusual conditions. The only affected rye plant was found at Spencerport, New York, on June 23, 1921, in an area in a wheat field in which all of the wheat plants had been killed with take-all.

*Agropyron repens* was the only wild grass found affected with take-all. In infected areas in wheat fields this grass was very commonly affected with take-all in at least three New York counties: Cayuga, Genesee, and Monroe.

*Greenhouse Host Range Tests.* To determine the host range of take-all inoculation tests were made on sixty-two varieties of cereals and forty-eight species of grasses in the greenhouse. The cereals and grasses included in the test were planted on February 24, 1921. In the first part of this test a set of three five-inch pots were planted with seed of each variety of cereals or species of grass, using clean soil. No inoculum was placed in the soil of one pot of each set, but inoculum consisting of a mixture of four pure culture isolations of the causal organism was placed in the soil of the other two pots at the time of planting. The pure cultures were obtained from ascospore isolations, the inoculum being produced in quantity by growing the fungus in pure culture on steamed wheat in Erlenmeyer flasks. About ten of these wheat kernels were mixed with the soil before planting. Ten cereal seeds or from twenty-five to fifty grass seeds were planted in each pot.

At the end of ten and a half weeks nearly all of the wheat plants in the artificially inoculated pots were stunted, and 34.6 per cent had died. The discoloration at the base of the stem ranged from slight to that produced by fully formed plate mycelium. As shown in table 1, the percentage of killed plants varied greatly with the different varieties. No symptoms of discoloration or marked stunting of the plants were found for rye, oats, barley, corn, or most of the species of the grasses. However, a few of the species of grasses showed marked symptoms of stunting and discoloration.

TABLE 1  
*Inoculation test on cereals*

Variety (Wheat unless otherwise specified)	Percentage of plants dying during first 10½ weeks of growth		Total number of heads formed per pot		Relative number of perithecia on plants at maturity in inoculated pots	Degree of infection at maturity
	Inoculated pots	Uninocu- lated pots	Inoculated pots	Uninocu- lated pots		
Kanred Ks 2401	34	0	0	3	Many	Heavy
Kanred P 1066	37	0	1	11	Many	Moderate
Kanred P 1068	41	0	1	4	Moderate	Moderate
New Malakoff	22	0	Dead	+	Moderate	Very heavy
Turkey Red	70	0	0	2	Moderate	Moderate
Kharkof	53	0	0.5	4	Moderate	
Forward	22	0	1.5	4	Moderate	Heavy
Georgia Bluestem	67	0				Moderate
Red Cross	63	0	0	3	Many	Heavy
Fultz	26	11	0	0	Many	Heavy
Number 8	14	0				Heavy
Poole	56	0	0.5	4	Many	
Red Wave	10		0.5	0	Many	Heavy
Red May	71		0.5	+	Many	Moderate
Fulcaster	11	25	2	2	Many	Moderate
Gypsy	22	0	0	5	Many	Moderate
Reliable	11	0	0	5	Many	Heavy
Red Rock	0		0.5	3	Many	Heavy
Velvet Chaff	25	0	0	5	Many	Moderate
Crails Fife	13	0	0	0	Moderate	Heavy
Imperial Amber	22		0	0	Many	Heavy

TABLE 1 (Continued)

Variety (Wheat unless otherwise specified)	Percentage of plants dying during first 10½ weeks of growth		Total number of heads formed per pot		Relative number of perithecia on plants at maturity in inoculated pots	Degree of infection at maturity
	Inoculated pots	Uninocu- lated pots	Inoculated pots	Uninocu- lated pots		
Number 6	59	0	0	1	Many	Heavy
Dawson Golden Chaff	31		0	4	Many	Heavy
Klondyke	25		0	0	Many	Moderate
Seneca Chief	13	0	1	3	Moderate	Moderate
S. S. Longberry	12	0	0	0	Many	Heavy
Genesee Giant	22	0	0	2	Many	Heavy
Early Arcadian	6		All dead		Many	Very heavy
Washington No. 108	0	0	1.5	15	Many	Heavy
O. A. C. No. 104	9	0	0	4	Many	Heavy
Little Club C. 1.4066	25	10	0.5	1	Many	Heavy
Bearded Club	19	0	11	8	Many	Moderate
Coffi	6	0	0.5	6	Many	Heavy
Marquis	27	0	0	8	Many	Heavy
Abynesia Purple Kernel	16	0	1.5	10	Many	Heavy
Coffee	24	0	0.5	12	Moderate	Moderate
Veivet Don	93	0	0.5	9	Many	Very heavy
Marovani	60		0.5	12	Many	Heavy
Belotonka	44	0	3	12	Many	Heavy
Arnautka C. I. 1493	71	0	Dead	7	Many	Very heavy
Acme	89	0	Dead	8	Many	Very heavy

TABLE 1 (Continued)

Variety (Wheat unless otherwise specified)	Percentage of plants dying during first 10½ weeks of growth		Total number of heads formed per pot		Relative number of perithecia on plants at maturity in inoculated pots	Degree of infection at maturity
	Inoculated pots	Uninocu- lated pots	Inoculated pots	Uninocu- lated pots		
Mindum	67	0	0	8	Few	Heavy
Poulard	53	0	1	10	Many	
Polonicum	29	0	2	12	Moderate	Moderate
Black Persian	59	0	0	10	Many	Very heavy
White Bearded Spelt	12	10	5	15	Moderate	Moderate
Black Bearded Spelt	21		3	8	Moderate	Moderate
Einkorn	60	0	3.5	13	Few	
Emmer C. I. 3686	60	0	0	8	Many	Heavy
Black Winter Emmer	17	0	0	5	Many	Moderate
Alaska Branched	0	0	0	10	Many	Very heavy
Pelesier	53	0	1	15	Many	Heavy
Khapli	36		0	11	Many	Heavy
Wild Wheat of Palestine	61	0	0	5	Many	
Barley 6 row	0	0	+	+	Many	Moderate
Malay Beardless	0	0	2	10	Very many	Moderate
Barley (C. I. 1176						
Oderbrucker	6		3	15	Very many	Moderate
Barley (C. I. 537)						
Oats	0	0	12	14	None	None
Rye Winter	10	0	0.5	1	Very few	Slight
Rye, Rosen	0	0	—	—	None	None
Field corn	0	0	—	—	None	None
Sweet corn						

Perithecia had formed in ten and one-half weeks after planting on the following plants in the artificially inoculated pots: winter wheats—Dawson Golden Chaff, Number 6 Junior, Seneca Chief, O. A. C. No. 104: spring wheats—Little Club, Einkorn, Velvet Don: grasses—*Agropyron repens*, *Bromus madritensis*, *Elymus virginicus*. Fully developed ascospores were found in the perithecia on Seneca Chief and O. A. C. No. 104 wheat.

The time taken by the plants to reach maturity varied from about four months for the spring cereals and the annual grasses, to seven months for the winter cereals and many of the perennial grasses.

The grains in the heads of the wheat plants growing in soil infected with the fungus were always very badly shriveled. The total grain produced by such plants weighed less than five per cent of the weight of that in heads of normal plants.

The relative number of perithecia on the affected plants varied from none to many. The term few means less than ten perithecia per culm; moderate, from ten to forty perithecia; and many, more than forty perithecia. Over one hundred perithecia were counted on single culms of Number 6 Junior, Velvet Don, and Red Rock wheat.

In the greenhouse no variety of the many types of wheat showed any marked degree of resistance to take-all, but the limitations of such experiments must be considered.

The three barley varieties tested showed no symptoms of take-all during the first ten and a half weeks of growth, but at the end of four months, when the barley was mature, the mycelial plate was even more pronounced than on wheat, and perithecia were as numerous as on any wheat variety. The characteristic stunting of the wheat plants seemed to be lacking in the case of the barley.

During the first four months of growth no symptoms of the disease appeared on rye. After six months, however, an indistinct plate mycelium and a few perithecia containing typical ascospores had developed on plants of Rosen rye. The results of inoculating forty-eight species of grasses are given in table 2.

Since the most important rôle of the grasses would be as carriers of the disease the chief object of this test was the determination of the grasses on which perithecia were produced. In order to determine this numerous culms of each species of grass were picked to pieces under the binocular, and where perithecia were present (Table 2) the presence of typical ascospores was demonstrated.

TABLE 2

*Inoculation tests on grasses. Condition of grasses from five to five and one-half months after planting seed in soil infested with O. cariceti.*

Name of grass	Presence of mycelium	Relative number of perithecia on plants in inoculated pots	Degree of infection
<i>Agropyron caninum</i> (L.) Beauv.....	Heavy	Many	Moderate
“ <i>cristatum</i> J. Gaert.....	Slight	Few	Moderate
“ <i>intermedium</i> Beauv.....	Heavy	Many	Heavy
“ <i>repens</i> (L.) Beauv.....	Heavy	Many	Very heavy
“ <i>smithii</i> Rydb.....	Slight	Few	Slight
“ <i>tenerum</i> Vasey.....	Heavy	Many	Moderate
<i>Agrostis alba</i> L.....		0	0
“ <i>canina</i> L.....		0	0
<i>Alopecurus pratensis</i> L.....		0	0
<i>Arrhenatherum elatius</i> (L.) Beauv...		0	0
<i>Anthroxanthum odoratum</i> L.....		0	0
<i>Avena fatua</i> L.....		0	0
<i>Briza maxima</i> L.....		0	0
<i>Bromus arvensis</i> L.....	Slight	Few	Slight
“ <i>ciliatus</i> L.....	Slight	Few	Slight
“ <i>erectus</i> Huds.....	Slight	Moderate	Moderate
“ <i>hordeaceus</i> L.....		0	0
“ <i>inermis</i> Leyss.....		0	0
“ <i>madritensis</i> L.....	Slight	Moderate	Slight
“ <i>japanicus</i> Thunb.....	Moderate	Moderate	Moderate
“ <i>racemosus</i> L.....	Moderate	Moderate	Moderate
“ <i>secalinus</i> L.....	Moderate	Many	Slight
“ <i>sterilis</i> L.....	Moderate	Many	Moderate
“ <i>tectorum</i> L.....	Moderate	Many	Moderate
“ <i>villosus</i> Forsk.....		0	0
<i>Dactylis glomerata</i> L.....		0	0
<i>Echinochloa crusgalli</i> (L.) Beauv....		0	0
<i>Elymus australis</i> Scribn. & Ball....	Moderate	Many	Moderate
“ <i>canadensis</i> L.....	Heavy	Many	Heavy
“ <i>virginicus</i> L.....	Moderate	Many	Moderate
<i>Festuca elatior</i> L.....	Slight	Few	Slight
“ <i>heterophylla</i> (Lam.) Hack..		0	0
“ <i>ovina</i> L.....		0	0
“ <i>pratensis</i> Huds.....		0	0
“ <i>rubra</i> L.....		0	0
<i>Holcus lanatus</i> L.....		0	0
<i>Hordeum jubatum</i> L.....	Heavy	Many	Heavy
“ <i>murinum</i> L.....	Slight	Few	Slight
“ <i>pusillum</i> Nutt.....		0	0



TABLE 2 (Continued)

Name of grass	Presence of mycelium	Relative number of perithecia on plants in inoculated pots	Degree of infection
<i>Hystrix patula</i> Moench.....	Moderate	Many	Heavy
<i>Lolium perenne</i> L.....		0	0
“ <i>temulentum</i> L.....	Slight	Few	Slight
<i>Phalaris arundinaceae</i> L.....	Moderate	Many	Moderate
<i>Phleum pratense</i> L.....		0	0
<i>Poa compressa</i> L.....		0	0
“ <i>pratensis</i> L.....		0	0
<i>Sataria glauca</i> (L.) Beauv.....		0	0
<i>Triodia flavus</i> (L.) Hitchc.....		0	0

The number of perithecia on each culm of the various species ranged from one to three in the case of *Festuca elatior*, to as high as one hundred and eight found on one culm of *Agropyron repens*. The terms, few, moderate, and many given in table 2, have the same meaning as in table 1.

The presence of mycelium refers to discoloration at the base of the culm of the infected plants. No definite plate of mycelium was found in the case of the grasses between the inner leaf and the culm. Rather numerous darkened strands of hyphae were formed in and between the several leaf sheaths.

A second series, in which five cereal varieties and fifteen grass species were used, was planted at the same time as the first in unsterilized soil taken from a diseased spot in a field and mixed with bits of diseased straw. The conditions under which this series was grown were the same as those of the first, except as stated. These plants developed typical take-all symptoms later in their growth than did those grown in soil infested with a pure culture of *O. cariceti*. However, at maturity the plants of the second series very closely resembled those grown in artificially inoculated soil in the degree of stunting, the presence of darkened tissue and plate mycelium, the relative number of perithecia, and the range of the species of grasses on which perithecia were found.

#### REPRODUCTION AND DISSEMINATION OF THE FUNGUS

##### FORMATION OF SPORES

Examination of field and greenhouse material, supplemented by examination of the fungus growing in pure culture on many kinds of media, confirms Lindau's statement (14) that no conidial form of the

fungus is definitely known. Healthy and diseased wheat plants have grown side by side for ten months in pots set in the soil of a greenhouse bench without the healthy plants becoming infected. If a conidial stage exists, it apparently plays no rôle in the dissemination of the fungus.

Sexual spores, however, are produced abundantly. From one to more than a hundred perithecia were formed on almost every diseased culm of wheat examined. Perithecia containing differentiated ascospores were found on winter wheat as early as June 5, from four to six weeks before the maximum number had been produced. In June and July the ascospores appear to be immature: they fail to germinate, they are from 10 to 20 $\mu$  shorter than those examined later in the year, and they are rarely, if at all, septate. Ascospores formed on winter wheat in June and July of 1920 failed to germinate when tested in July and August. Ascospores from the same source kept under field conditions, germinated profusely on October 20, November 10, November 16, and December 3, 1920, and on January 19 and March 26, 1921, but failed to germinate on August 20, 1921. This would indicate that under field conditions the ascospores can germinate and may infect their hosts either during the fall or the following spring.

#### METHOD OF DISSEMINATION OF THE FUNGUS

The literature gives little information as to how *Ophiobolus* is carried from one locality to another. It has been pointed out by Waters (31) that infective material may be carried for a short distance on the feet of animals and on cultivators. Observations made thus far in the field indicate that the pathogene is disseminated commonly by infected straw in manure. McAlpine (16) believes that dust or wind-blown soil is responsible for much of the distribution of the fungus in parts of Australia, although this probably would occur less frequently in the humid regions of the United States. Waters (31) states that in New Zealand "no instance could be found of the rapid spread of take-all from crop to crop through the air like rust." Since most of the ascospores are produced near or even below the ground level, it does not seem likely that wind is an important factor in the dissemination of the fungus under our conditions.

Many growers rake over the stubble after the binder. The material thus collected goes with the bulk of the harvest to the thresher. The fungus may thus be spread either with the thresher or with improperly cleaned seed.

The series of tests tabulated below were planned to determine whether seed, soil, or plant residue acted as a carrier of the fungus. The plantings were made at different dates, as given in the tabulations. From ten to fifteen seeds of Number 6 Junior or Dawson Golden Chaff were

planted in each pot. The pots which were five-inch size, were set four inches in the soil of a greenhouse bench where they remained throughout the test. Unsterilized bench soil was considered as being clean soil. The infested soil used came from a spot in a field in which diseased plants had been grown, and was carefully screened in order to remove bits of infected tissue that might have been present. The inoculum in every case was mixed with the soil when the seed were planted.

*Shriveled seed from diseased plants. Clean soil. No prepared inoculum*

July 31, 1920	2 pots	Plants exhibited no symptoms of infection and grew to maturity producing normal heads.
September 13, 1920	1 pot	
October 4, 1920	4 pots	

*Shriveled seed from diseased plants. Soil from infected spot in field*

July 31, 1920	4 pots	All plants stunted and many killed.
September 13, 1920	2 pots	Perithecia on all. Infection heavy.
October 4, 1920	5 pots	

*Clean seed. Clean soil. No prepared inoculum*

July 31, 1920	2 pots	Plants exhibited no symptoms of infection and grew to maturity producing many normal heads.
September 13, 1920	2 pots	
October 4, 1920	2 pots	
Mar. 26, 1921	3 pots	

*Clean seed. Soil from infected spot in field. Soil had been stored indoors since July, 1920*

October 4, 1920	5 pots	Plants in two of the five pots diseased, those in the other three pots showing no sign of infection.
March 26, 1921	4 pots	Plants somewhat stunted but exhibiting no typical symptoms. No perithecia found.

*Clean seed. Clean soil. Bits of diseased straw containing perithecia used as inoculum*

July 31, 1920	2 pots	All plants stunted and killed, exhibiting typical symptoms. Many perithecia. Infection very heavy.
March 26, 1921	3 pots	

*Clean seed. Soil from diseased spot in field. Bits of diseased straw containing perithecia used as inoculum*

September 13, 1920	4 pots	All plants stunted and killed, exhibiting typical symptoms. Many perithecia. Infection very heavy.
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These tabulations indicate that seed from diseased plants do not act as carriers of the disease; that carefully screened soil from infected spots in the field acts as inoculum for a time, but that such soil when stored indoors for a period of eight months no longer contains infective material

and that bits of straw containing perithecia are the most virulent inoculum used, the virulence of this inoculum being undiminished at the end of eight months as was the case with the soil. Further, since the healthy plants grew side by side with diseased plants throughout the test without becoming infected, it seems that the soil was the principal source of inoculum.

#### SOME FACTORS INFLUENCING THE GROWTH OF THE FUNGUS

Many investigators have attempted to influence the growth of the fungus by the application of various fertilizers and other substances. Lime has generally been reported as greatly increasing the amount of disease (2). Superphosphate of lime has been reported as increasing (2), as not checking (18), and as decreasing (10) the growth of the fungus. Copper sulphate in some cases has had no effect (9), and in other cases has caused a marked reduction in growth (3). The application of from 100 to 1000 pounds of iron sulfate per acre has generally checked the growth of the fungus (5, 3). From these more or less conflicting reports, it seems that the addition of alkali favors the fungus, while acid seems to retard it.

To test the effect of certain fertilizers in a preliminary way the following experiment was run in the greenhouse: Twenty-five five-inch pots were filled with clean soil and were inoculated at the same time and under the same conditions as those of the first part of the host-range test, except that five kernels of Number 6 Junior wheat were planted in each pot. The twenty-five pots were divided into five series of five pots each. The first series of pots received no fertilizer, while each of the other four series received one of the following fertilizer treatments:

1. One gram of sodium nitrate per pot, applied by dissolving in 100 cc. of water which was poured over the soil.
2. One-half gram of acid potassium phosphate (diabasic) per pot. Applied by mixing with soil.
3. One gram of sulphur per pot. Applied by mixing with soil.
4. Three grams of hydrated lime per pot.

After seventeen weeks of growth, no marked differences were found among the different series. Later, the series to which lime had been added was the first on which perithecia were found, and this series had more killed plants (15.7 per cent) than the sodium nitrate series (13.6 per cent), the acid potassium phosphate series (5 per cent), or the sulphur series (none) and had less dead plants than the series to which no fertilizer had been added (25 per cent).

After eight months of growth, the plants in all of the series except the one to which sulphur had been added were heavily infected, were

badly stunted, and had produced no heads. Even the plants in the sulphur series were heavily infected, but those in three of the pots succeeded in producing several heads. If anything, acidity of soil seemed to have delayed the attack of the fungus or lessened its severity.

In order to further test this point, the fungus was grown in culture on an acidity series consisting of potato agar having nineteen degrees of acidity ranging from 3.2 to 9.6 pH, and bacto-cornmeal agar having ten degrees of acidity ranging from 3 to 9.2 pH. The degree of acidity of the media was determined by the colorimeter method as recommended by Clark (4).

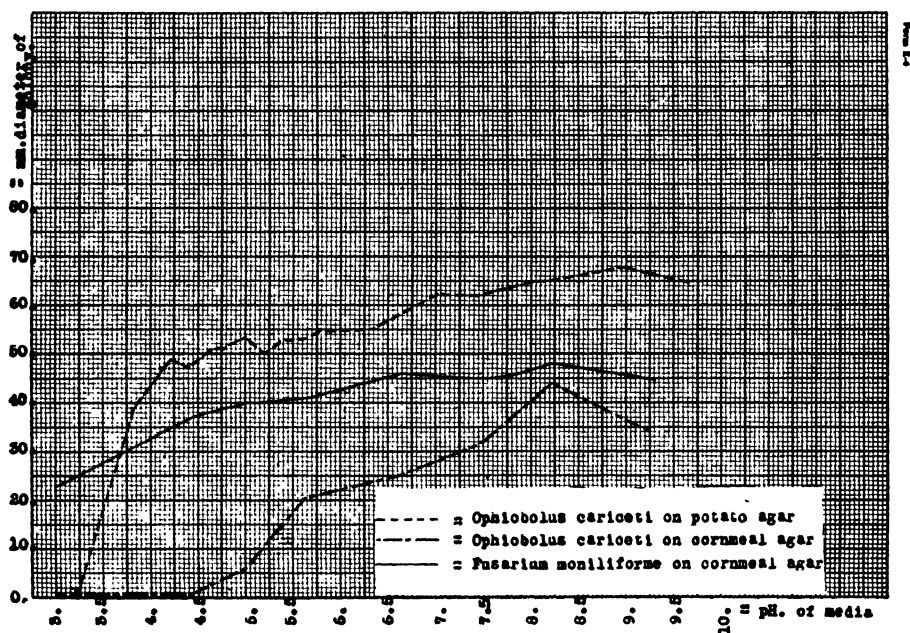


FIG. 3. RATE OF GROWTH OF *Ophiobolus cariceti* AND *Fusarium moniliforme* ON AGAR HAVING VARYING DEGREES OF ACIDITY

Cultures of *O. cariceti* from three sources, in comparison with one strain of *Fusarium moniliforme* Sheld., were grown on the media in total darkness at room temperature (15 to 20° C.) The average growth of the three strains of *O. cariceti* and the growth of the single strain of *F. moniliforme* at the end of eight days are shown in figure 3.

The results indicate that the fungus requires alkalinity for optimum growth, and may explain why additions of alkaline substances to the soil have been reported as favoring the fungus while additions of acid-forming substances decrease the amount of infection. The writer now has under way several series of greenhouse and field tests to determine the effect of soil acidity on the resistance of the hosts to the fungus.

Several investigators (2, 7) point out that excessive soil moisture is favorable to the growth of the fungus. For this reason, careful observations were made of the relation of soil moisture to the severity of the disease. In most fields examined, apparently there was little or no difference in the amount of infection on high and low ground, although in a few fields the infection appeared to be very much heavier on the lower and wetter land.

#### CONTROL

Many suggestions appear in the literature concerning the control of take-all, among the most common of which are: eliminating the fungus by burning the diseased stubble and by eradicating wild grass and volunteer grain which act as hosts (16, 25, 28); practicing long rotations, using crops believed to be immune (16, 23, 31); retarding infection by the addition of certain substances to the soil such as phosphates and sulphates, which either check the growth of the fungus or so strengthen the cereal plants that they may become resistant (27, 5, 10); proper drainage of the soil (2, 7); escaping infection by the practice of late planting (28, 31); preventing infection by the use of resistant varieties (10, 21). The statements in regard to some of these methods are so conflicting as to place their value somewhat in doubt.

Rotation appears to be an important method of control, since in New York State it was observed that nearly every field in which more than five per cent of the plants were killed by take-all had been planted to two or more successive crops of wheat. A rotation, to be effective, should have wheat not oftener than every four or five years, since the fungus can live in the stubble for at least a year, and several years of cropping may be required for the fungus to die out on volunteer grain or other hosts.

Diseased wheat stubble is the principal carrier of the take-all fungus. Therefore, straw containing plants broken or cut off low enough to make them carriers of the organism, should not be returned to the land in manure or in any other way for at least three years preceding the planting of wheat. It was observed that wheat straw from diseased fields, when applied in manure during the fall and winter of 1920-21, acted as a direct carrier of the fungus.

Wheat seed should be thoroughly cleaned before planting. The mycelium does not seem to be carried inside the seed, nor do the spores seem to be carried on the seed coat, but bits of straw containing perithecia may become mixed with the threshed grain and the pathogene might then be carried with the seed. It would be well not to lime the soil before planting wheat, since acidity retards and alkalinity promotes the growth of the take-all fungus. Other suggested methods of control are late planting and the use of resistant varieties, although definite recommendations cannot be made on these two points.

## SUMMARY

1. The take-all disease of cereals and grasses caused by the fungus *Ophiobolus cariceti* (B. & Br.) Sacc. was first found in New York in 1920. A survey made in 1921 demonstrated that the disease was present in nearly one half of the winter wheat fields of sixteen counties in the west central part of the state.

2. The disease was comparable in severity to that described in Australia and Europe. The average damage in the seventy-eight fields found infected in 1921 was about two per cent, and the maximum damage in any one field was about twenty per cent.

3. The most typical symptom of the disease is a dwarfing of the host, which includes a reduction in height, in the number of tillers, in the number of heads, and in the size and amount of grain produced. The yield of an infected wheat plant was, on the average, about one per cent that of a healthy plant.

4. The fungus is confined to the roots and the lower internodes of the host, where a pronounced discoloration occurs. A typical plate of mycelium is found between the leaf sheath and the culm, and perithecia are produced in abundance, more than one hundred having been found on single culms of wheat and of *Agropyron repens*.

5. As the results of inoculations in the greenhouse, typical perithecia were produced on wheat, barley, rye, and one or more species of the following genera of wild grasses: *Agropyron*, *Bromus*, *Elymus*, *Festuca*, *Hordeum*, *Hystrix*, *Lolium*, *Phalaris*.

6. None of the fifty-four varieties of wheat tested in the greenhouse showed any marked degree of resistance to take-all. These varieties included representatives of the following species of the genus *Triticum*; *aestivum*, *compactum*, *turgidum*, *durum*, *dicoccum*, *spelta*, *polonicum*, and *monococcum*.

7. The causal organism was isolated and grown in pure culture on numerous media. Typical perithecia have been produced in pure culture.

8. In the present investigation, seed from diseased plants did not act as carriers of the disease. Screened soil from infected spots in fields acted as inoculum for several months, but at the end of eight months the soil which had been kept in the laboratory was not a carrier of the disease. Bits of infected straw containing perithecia were very effective inocula, and in this case the virulence of the organism had not decreased at the end of eight months.

9. *O. cariceti* requires a condition of alkalinity for optimum growth. On cornmeal agar, growth begins at about 4.5 pH. and increases gradually to 8.1 pH, the point at which maximum growth occurs.

10. In the present investigation, the relation of *O. cariceti* to take-all was determined by the established rules of proof.

11. The most promising methods of control seem to be: the practice of four to five year rotations; eradicating wild grass and volunteer grain which may act as hosts; discontinuing the practice of returning wheat stubble in manure for three years preceding the planting of wheat; cleaning the wheat seed thoroughly to remove all bits of straw which might carry perithecia; and discontinuing the practice of liming the soil before planting wheat.

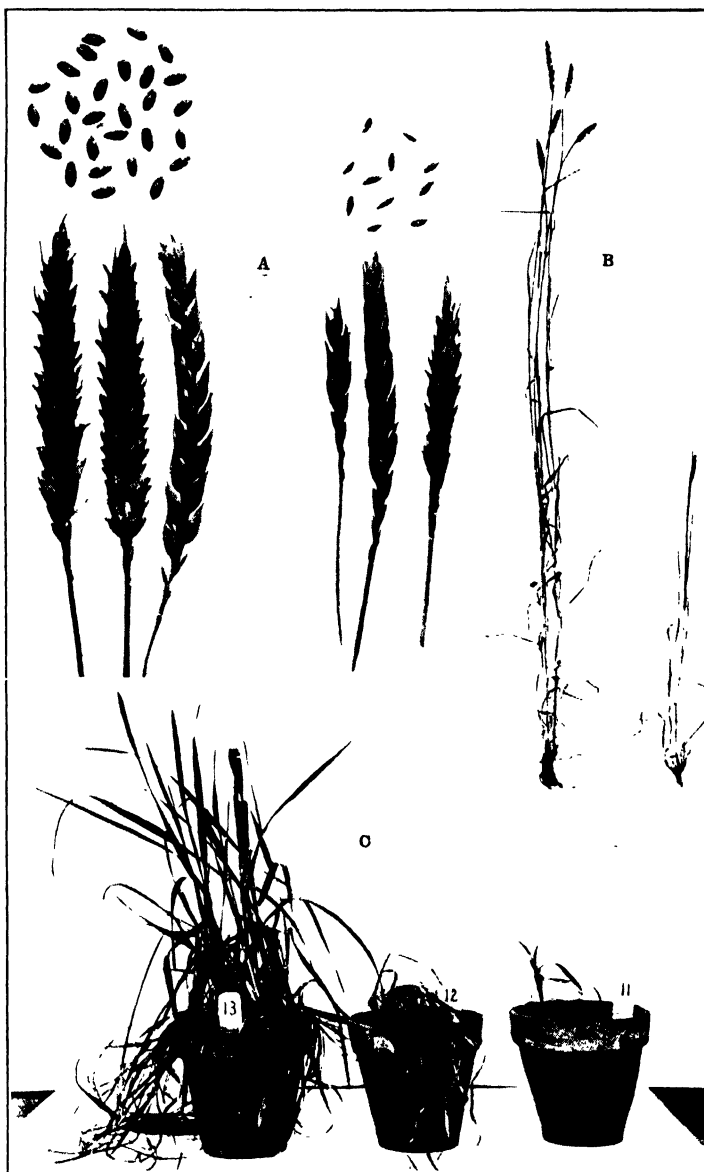
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TAKE-ALL DISEASE OF CEREALS AND GRASSES

FIG. A. Heads taken from healthy (left) and take-all infected (right) wheat plants.

FIG. B. Healthy and take-all infected wheat plants. On left, healthy plant with five heads, height 40 inches. On right, diseased plant with one shriveled head, height 18 inches.

FIG. C. Healthy and diseased plants after four months' growth.

Pot 13. Check.

Pot 11-12. Diseased straw mixed with soil at planting time.





TAKE-ALL DISEASE OF CEREALS AND GRASSES

FIG. A. Two wheat plants (height 16 in.) infected with take-all taken from a field at harvest time. The marked stunting of the plants is characteristic of this disease.

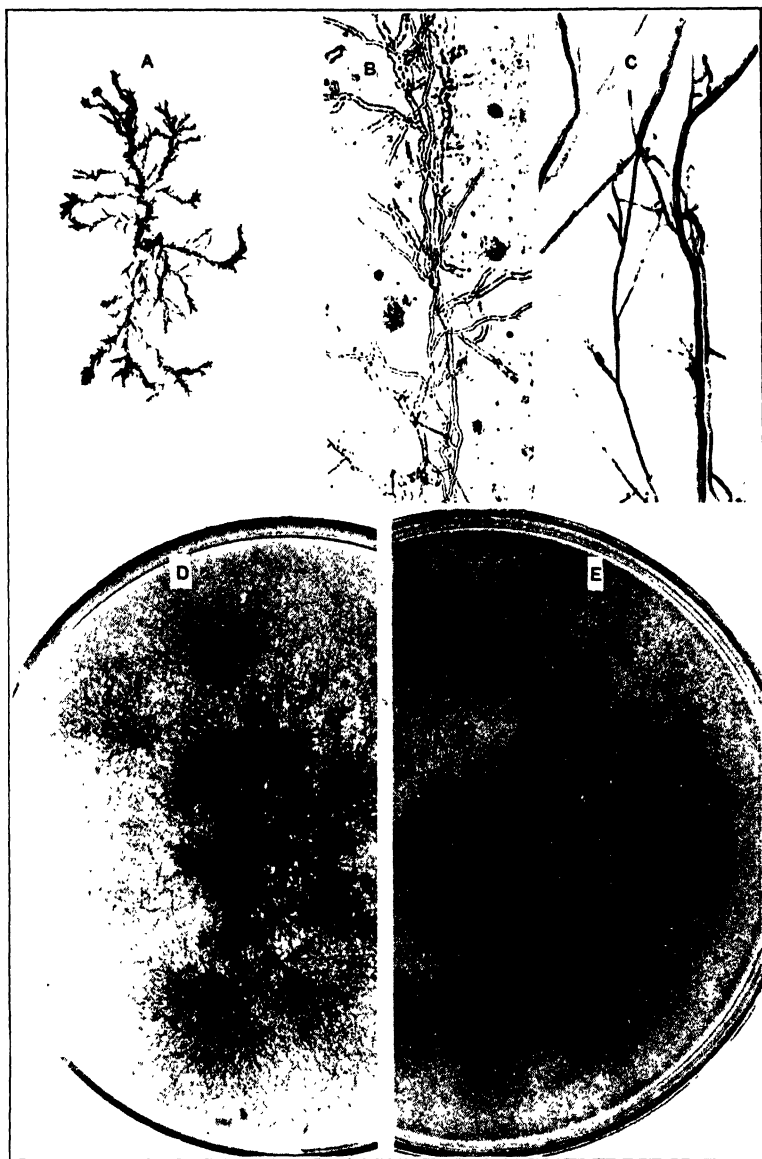
FIG. B. Base of wheat plant infected with take-all showing beaks of perithecia protruding through the leaf sheaths.  $\times 4$

FIG. C. A portion of the mycelial plate found about the base of the culm.  $\times 160$

FIG. D. Longitudinal section through the outer leaf sheath of a wheat plant showing structure and contents of perithecia. Note the beaks of the perithecia protruding obliquely through the leaf sheath.  $\times 48$

FIG. E. Asci and ascospores.  $\times 160$





## TAKE-ALL DISEASE OF CEREALS AND GRASSES

FIG. A. Thirty days old culture of *O. cariceti* on 0.2 per cent dextrose agar. x 9

FIG. B. Characteristic rhizomorphic strands of mycelium as found on 2 per cent dextrose agar after 30 days' growth. x 450

FIG. C. Characteristic ribbon-like rhizomorphic bands of mycelium as found on 2 per cent dextrose agar after 30 days' growth. x 450

FIG. D. Thirty days' old culture of *O. cariceti* on 2 per cent dextrose. x 9

FIG. E. Thirty days' old culture of *O. cariceti* on potato agar. x 9



# STUDIES ON CORN RUST

GEORGE F. WEBER<sup>1</sup>

WITH THREE FIGURES IN THE TEXT

## INTRODUCTION

In connection with studies on corn rust (*Puccinia sorghi* Schw.) conducted at the University of Wisconsin during the year 1919-1920, data were obtained particularly on: (1) the relation of temperature to germination of urediniospores; (2) the relation of temperature to infection by urediniospores; (3) mode of host penetration in uredinial infection; (4) overwintering of urediniospores; (5) relative susceptibility of corn species. Corn seedlings were grown in the greenhouse in eight-inch pots and in flats six inches deep. Urediniospores were obtained from infected plants in the greenhouse, except when otherwise stated. Inoculations were made by rubbing the corn leaves with wet absorbent cotton after which the spores were gently applied with a scalpel or platinum loop. All inoculated plants were incubated in a moist chamber for thirty-six hours immediately following inoculation. In the course of the studies it was noted that when urediniospores were shaken from pustules their percentage of germination was higher than when the spores were removed with a scalpel. Hence in order to collect uninjured spores of the same stage of maturity for the experiments, a medicine dropper was used as follows: A droplet of water was squeezed from the dropper and gently stroked over the top of the pustule, the mature spores were picked up readily by the surface of the drop.

## EXPERIMENTAL STUDIES

### THE RELATION OF TEMPERATURE TO GERMINATION OF UREDINIOSPORES

In germinating rust spores Johnson (3) found no uniformity between the temperatures of 7° and 25° C. Melhus (6) determined the optimum temperature for the germination of urediniospores of *Puccinia sorghi* Schw. at 18° C. Mains (5) found 15°-18° C. to be the optimum and 25° C. the maximum temperatures for urediniospore germination.

<sup>1</sup>The writer wishes to express his sincere appreciation to Dr. A. G. Johnson for suggesting the investigation of this problem and for assisting in the preparation of the manuscript; to Dr. J. G. Dickson for the many valuable suggestions and helpful criticisms during the progress of the work; and to Mr. J. R. Holbert for furnishing the seed of the different kinds of corn used in these experiments and for access to his experimental plats for the purpose of making observations.



Mature urediniospores collected by the writer in the manner stated above were placed in hanging drops on the covers of petri dishes, the bottoms of which were covered with water and placed at different temperatures in an Altmann graduated incubator. At the end of twenty-four hours counts were made of germinated and ungerminated spores. The average percentages of germination of twelve series are given in table 1 and shown graphically in figure 1. In each series from four to twelve drop cultures were observed for each temperature.

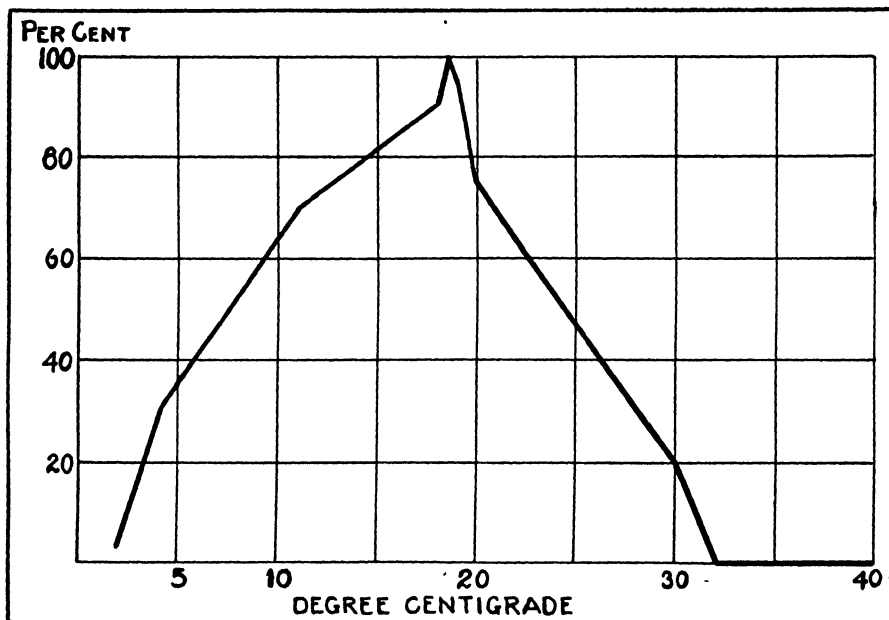


FIG. 1. GRAPH SHOWING PERCENTAGES OF GERMINATION OF UREDINIOSPORES OF CORN RUST AT DIFFERENT TEMPERATURES

TABLE 1

*Temperatures of incubation and percentages of germination of urediniospores of corn rust after 24 hours*

Temperature in degrees C.....	4	8	12	16	17	18	20	25	30	34	40
Germination.....	3	30	70	90	100	95	75	60	20	0	0

It will be noted that the highest percentage of germination resulted at 17° C., and that germination diminished rapidly toward the lower and higher temperatures.

At 8° C. the germ tubes were short, thick, very definite in outline and appeared turgid. At the tips of the germ tubes the protoplasm was denser than toward the spore. In contrast with this at 30° C. the germ tubes were long, narrow, hyaline, thin-walled, without visible protoplasm, and showed some lack of turgor.

## THE RELATION OF TEMPERATURE TO INFECTION BY UREDINIOSPORES

A series of corn seedlings fifteen days old were inoculated and placed in temperature chambers regulated at 8°, 14°, 18°, 25° and 32° C. respectively. The humidity was not controlled but it was kept close to saturation. The results tabulated in table 2 are given in percentage of infection after twelve days according to a scale of comparison adopted by the Office of Cereal Investigations, United States Department of Agriculture, and published by Durrell and Parker (2).

TABLE 2

*Percentage of infection on corn plants inoculated with urediniospores of Puccinia sorghi and incubated at different temperatures for twelve days*

Temperature in degrees C.....	8	14	18	25	32
Percentage of infection.....	29	50	90	20	0

The data in table 2 show that the highest percentage of infection took place at 18° C, and that infection gradually decreased toward the higher and lower temperatures respectively. The urediniospores did not germinate at 32° C., and at 30° C. they showed only a slight tendency toward germination, but at this temperature they did not infect the corn plant. Infection took place at 28° C. but the fungus did not develop well. The rate of development of the fungus within the host was most rapid at 20° C.; at lower temperatures its growth was slower.

## MODE OF HOST PENETRATION IN UREDINIAL INFECTION

The second and third leaves of corn seedlings, ten days old, growing in 8-inch pots, were inoculated with mature urediniospores. The inoculated plants were placed in a moist chamber and, successively after twenty-four, thirty-six and forty-eight hours, pieces 1 cm. square were cut from the inoculated leaves. These pieces were placed in a solution of equal parts of glacial acetic acid and 95 per cent alcohol for twenty-four hours. By this method the cells were killed, fixed and cleared. They were then stained *in toto* with Pianese IIIb stain, as used by Vaughan (7), and examined under the low power of the microscope. The host tissue stained green and the fungus rose.

When the surface of the leaf was moist the germ tubes extended in all directions, when dry they showed a marked tendency to follow the sunken furrows between the epidermal cells. It was also shown in these studies that there was no attraction of the germ tube toward the stomata, although one was never observed growing across the stomatal opening without entering. Usually an appressorium was formed over the stoma, often almost covering it. Then it sent out a slender thread-like hypha into the stomatal chamber, which developed into mycelium (Fig. 2a). However, not all germ tubes develop appressoria before entering the stomata (Fig. 2b). If the stomata were open the germ

tubes entered immediately. The mycelium was developed in the intercellular spaces in contact with the parenchymatous cells of the host.

#### OVERWINTERING OF UREDINIOSPORES

In reviewing the literature on corn rust no data, based on experimental evidence, were found concerning the overwintering of urediniospores. However, Carlton (1) observed that the urediniospores of *Puccinia sorghi* Schw. did not remain viable over winter. Kellerman

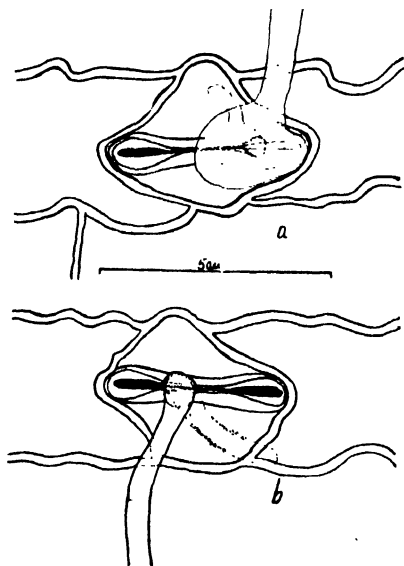


FIG. 2. CORN RUST ENTERING STOMATA OF CORN LEAF.

a. Hypha from urediniospore formed appressorium over one end of stoma and penetration tube from appressorium entered opening of stoma.

b. Hypha from urediniospore entered opening of stoma without forming appressorium.

(4) stated that surviving urediniospores carried the disease over winter. This lack of data prompted the writer to conduct a series of experiments during the winter of 1919-1920, at Madison, Wisconsin, for the purpose of determining the viability of urediniospores from the time of their maturity in September until May of the following year. Rusted corn leaves were collected before frost and carefully dried at room temperature. They were then placed at ten different stations under somewhat varied environmental conditions. These stations were designated by the numbers 1-10. Stations 1 to 4 were on the bare ground on the four sides of a hill, namely, north, east, south and west sides respectively. Stations 5 and 6 were on the north and south sides respectively of a greenhouse. Stations 7 to 9 were corn leaves suspended

at different heights from the ground on the side of a building. At station 10 the corn leaves were slightly covered with soil. All germination tests were made at 18° C. Germination tests were made just before the rusted leaves were placed at the different stations, October 1, 1919, and every two weeks thereafter until April 30, 1920. All the germination tests were made in distilled water by placing the spores in hanging drops in petri dish covers as previously described and incubating in the dark. The average percentages of germination at the different stations at the different times are given in table 3.

The results are given in percentage in all cases except where "S" follows the figure, in which cases the figure indicates the number of spores germinating on the whole petri dish cover. At first, 18 hours were sufficient for germination, but as the spores became less viable, 24 hours and finally 48 hours were required for the germination test.

TABLE 3

*The average percentages of the germination of urediniospores collected from the different stations from October 1, 1919, to April 30, 1920*

Date	Percentage germination of spores									
	Station									
	1	2	3	4	5	6	7	8	9	10
Oct. 1	100	100	100	100	100	100	95	100	100	100
Oct. 15	90	70	90	85	90	80	90	100	90	95
Nov. 1	90	40	85	70	70	60	60	95	75	75
Nov. 15	80	10	60	40	30	25	10	80	25	14
Dec. 1	75	3S	75	5	0	1	45	6	20	0
Dec. 15	50	1	20	5	5	2	5	1	5	1S
Jan. 1	5	0	2	0	7	5	0	0	1	1
Jan. 15	5	0	2	0	2	0	0	0	2	0
Feb. 1	0	1S	0	1	0	0	0	0	4S	0
Feb. 15	2S	0	0	0	0	0	0	0	0	0
Mar. 1	0	0	0	0	0	0	0	0	0	0
Mar. 15	0	0	0	0	0	0	0	0	0	0
Apr. 1	0	0	0	0	0	0	0	0	0	0
Apr. 15	0	0	0	0	0	0	0	0	0	0
Apr. 30	0	0	0	0	0	0	0	0	0	0

It will be noted that there was a high percentage of germination during the first 30 days, followed by a rapid decline in germination from 40 to 60 days after the first tests were made. Coincident with this decline in germination the normal appearance of the germ tubes changed. They became more hyaline, thin-walled, and showed some lack of turgor.

It will be seen from table 3 that good germination resulted up until December 1, 1919, when for the first time no germination was observed from certain stations. On January 1 the urediniospores from other stations showed no germination. On January 3 urediniospores were collected from the ten stations and used as separate inocula on twelve day old corn plants in the greenhouse. These corn seedlings were in the fourth leaf stage. From each collection a spore suspension was made and the third leaf of five different corn plants was inoculated in



FIG. 3. UREDINIA OF CORN RUST ON MESOCOTYLS OF DENT CORN FOLLOWING ARTIFICIAL INOCULATION OF GERMINATING KERNEL.

the usual way. These plants were incubated in a moist chamber at 20° C. for 48 hours and then removed to a greenhouse at 24° C. No infection resulted from any of these inoculations. The check plants similarly inoculated and incubated, except with fresh urediniospores, showed abundant uredinia containing mature spores. After eight days four other similar series of tests were conducted with the same results,

i. e., no infection except where fresh spores were used. The inoculated plants were kept under observation for 20 days. Hence it was evident that the spores were either dead or had lost their pathogenicity.

In connection with the overwintering studies, certain attempts were made to determine if soil or seed borne infection were at all possible. Corn seeds were germinated in moist chambers until the coleoptile and radicle were about one-half inch long. They were then submerged in a urediniospore suspension for five minutes and immediately planted two inches deep in sterilized soil. After four weeks the soil was washed from the corn roots and uredinia containing mature spores were found on the mesocotyls of the seedlings one and one-half inches below the surface of the soil. Figure 3 shows the uredinia on the mesocotyls of two of these corn seedlings.

While uredinia were obtained in this way, it was not shown that they could function in initiating rust infections on the above-ground parts. Under certain conditions, however, this might not be impossible.

#### RELATIVE SUSCEPTIBILITY OF CORN SPECIES

In order to determine the relative susceptibility of corn species it was found necessary to establish a strain of rust to be used throughout the series of experiments. This was done as follows: Rusted leaves had been collected by the writer from sixty-three different species and varieties of corn, grown in an experimental plat at Bloomington, Illinois, by J. R. Holbert. In preliminary experiments urediniospores from each of these collections were used to inoculate corn seedlings of the seven species and six varieties. For each set of inoculations the corn seedlings used were grown in greenhouse flats 16x24x4 inches. It was found that all of the spore collections behaved alike on each of the species and varieties tested. However, on the various species and varieties there was a rather constant range in susceptibility to the rust from the different sources. Hence no evidence of specialization in the rust was found.

On the basis of the above results urediniospores grown in the greenhouse on various species and varieties of corn were used in subsequent tests of relative susceptibility. In testing the relative susceptibility the following seven species of corn were used: *Zea everta* (pop); *Z. indurata* (flint); *Z. amyloacea* (flour); *Z. saccharata* (sweet); *Z. indentata* (dent); *Z. tunicata* (pod); and *Z. ramosa*. These corn species were grown in flats in the greenhouse as previously described. When the seedlings had developed their second leaves they were inoculated with fresh, mature, urediniospores developed on greenhouse plants. The inoculated plants were then placed in a moist chamber for twenty-four hours. They were then removed to a greenhouse at a temperature

of about 26° C. Five weeks later when the plants were about eighteen inches high, they had developed six additional leaves (the youngest was not fully unrolled) and secondary rust infection was general. At this time the relative abundance of rust on each species of corn was carefully noted according to the scale for estimating rust as used by the Office of Cereal Investigations, United States Department of Agriculture, and published by Durrell and Parker (2). The two first leaves that were inoculated were not included in taking the rust reading since they had all been inoculated, using artificial technique and consequently were all rather heavily rusted; in fact, some of them were dried up when rust ratings were taken. Nor was the youngest leaf included in the rust readings since, being only partly unrolled it was not old enough to have developed the rust. Hence only five leaves (third to seventh inclusive) were used in taking the rust percentage readings. Seven series of these inoculations were carried out and the readings similarly taken. The results from these seven series were averaged and the averages are given in table 4.

TABLE 4

*Average percentages of rust on the seven species of corn and their rank as to relative susceptibility*

Species	Average rust percentages	Rank as to susceptibility
<i>Zea. saccharata</i> (sweet).....	74	1
<i>Z. indurata</i> (flint).....	70	2
<i>Z. amylacea</i> (flour).....	65	3
<i>Z. indentata</i> (dent).....	58	4
<i>Z. ramosa</i> .....	44	5
<i>Z. tunicata</i> (pod).....	32	6
<i>Z. everta</i> (pop) .....	22	7

These data are in accord with general field observations particularly in regard to the greater susceptibility of sweet corn to corn rust.

#### SUMMARY

The results of the investigations herein reported indicate:

1. That the minimum, optimum, and maximum temperatures for germination of urediniospores of corn rust are 4°, 17°, and 32° C. respectively.
2. That the optimum temperature for infection is about 18° C. The minimum and maximum temperatures were not definitely determined but were found to be somewhat below 8° C. and 32° C. respectively.
3. That the germ tubes enter the stomata of the corn plant either with or without the formation of appressoria.

4. That, urediniospores did not overwinter in the vicinity of Madison, Wisconsin, during the winter of 1919-1920.

5. That, while there is no evidence of specialization on the part of the rust, there seems to be rather definite differences in susceptibility to the rust in the different species of corn.

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## BRIEFER ARTICLES

### ADDITIONAL HOSTS FOR BACTERIUM SOLANACEARUM

FREDERICK A. WOLF

One of the striking features exhibited by *Bacterium solanacearum* is its ability to attack a considerable number of species belonging to widely separated families. A total of about 50 species of host plants classified among 9 families have thus far been reported to be subject to attack. Attention was directed to this fact in a previous paper<sup>1</sup> from this laboratory wherein 13 species of plants hitherto not regarded as hosts exhibited well defined wilting or serious injury following inoculation with pure culture. This list has subsequently been enlarged to include castor bean (*Ricinus communis*)<sup>2</sup> and bean (*Phaseolus vulgaris*)<sup>3</sup> both of which have been noted to be naturally infected in Florida. These last reports furthermore include the results of successful artificial inoculations, under certain conditions, into cotton, vanilla, fuchsia, sunflower, garden pea, cowpea, and soybean.

During August 1921, my attention was directed to a wilting of soybeans (*Soja max*) dahlia (*Dahlia rosea*) and cosmos (*Cosmos bipinnatus*). The specimens of soybeans were sent from Columbus, N. C., by Mr. J. R. Sams and those of the other hosts were collected in a garden in Raleigh, N. C. In the case of soybeans, probably because of the woody nature of their stems and petioles, wilting is not a prominent symptom. Affected plants are dwarfed, however, and the foliage becomes prematurely dry and dead. In the case of both dahlia and cosmos, the tops of the plants show a sudden wilting and drooping. During the night, such plants may recover temporarily but after several days, the leaves shrivel, become dry, and remain attached to the plant, which ultimately dies.

No very marked discoloration of the vascular system, except near the base of the stem, was apparent in the case of any of the species. Upon microscopic examination of the xylem tissues, however, bacteria were found to be abundantly present well above the surface of the ground.

<sup>1</sup> Stanford, E. E. and Wolf, F. A. Studies on *Bacterium solanacearum*, Phytopath. 7: 155-165; fig. 1, 1917.

<sup>2</sup> Smith, Erwin F. Bacterial wilt of castor bean (*Ricinus communis* L.). Jour. Agr. Res. 21: 255-261, pls. 55-67. 1921.

<sup>3</sup> Smith, Erwin F., and McCulloch, Lucia. *Bacterium solanacearum* in beans. Science, n. s. 50: 238, 1919.

These bacteria were isolated from each host and invariably in pure culture by the following procedure. The surface of the stems was first sterilized in mercuric chloride. The stems were then cut off with a sterile scalpel and by means of a forceps the plant juice together with the bacteria was squeezed out at the cut end. A platinum loopful of this exudate was spread with a zigzag stroke over the surface of a hardened agar plate. After two days incubation, numerous colonies had developed on all plates. These colonies were well isolated especially near the end of the stroke. Because of their distinct opalescent blue color by oblique light and brownish color by transmitted light, these colonies gave indications that the causal organism was *Bacterium solanacearum*. They were accordingly compared with cultures of this organism isolated from wilted tobacco collected several weeks earlier near Washington, N. C., and with a strain recently isolated from wilted tomatoes from Raleigh, N. C. All were found to be similar in their ability to brown agar media and broths containing peptone. Further, no differences were apparent in fermentation studies with either dextrose, saccharose, lactose, maltose, glycerine, galactose, dextrine and mannite in bouillon consisting of 1 per cent Armour's peptone 0.3 per cent Liebig's beef extract and 0.5 per cent sodium chlorid. Cultures from strains became progressively more alkaline from an initial reaction of pH=7.2 with each of these carbon compounds, and none formed gas. No inoculations experiments were attempted.

In view of the fact that *Bact. solanacearum* has been repeatedly isolated in this laboratory and has been studied for a term of years, and that the strains from soybean, dahlia and cosmos are like those from wilted tobacco and tomatoes in the above mentioned cultural characters, which are identical with those of *Bact. solanacearum*, the diseased condition of soybean, dahlia and cosmos must have been brought about by this bacterial organism.

#### A LEAFSPOT DISEASE OF TOBACCO CAUSED BY PHYLLOSTICTA NICOTIANA E. AND E.

FREDERICK A. WOLF

In the course of studies of diseases affecting the foliage of tobacco, one of minor importance has been noted which apparently has not previously been made the subject of investigation. This disease has been observed for several seasons both upon seedlings in the plant bed and upon more mature plants in the field. It manifests itself by the formation of brownish zonate spots, irregular in outline, which vary in size from 1 to 10 mm. The spots are of the lightest shade of brown near the center and of the darkest zone near the margin. A border of pale green or yellowish green tissues surrounds the invaded dead tissues. Pycnidia are sparsely present in these dead tissues. They can be seen

with difficulty with low magnification because their color is so nearly that of the surrounding tissues. These pycnidia are thin walled, ostiolate and vary in diameter from 75 to 150 $\mu$ . The conidia are hyaline, an occasional one is one-septate, and they measure 6-10  $\times$  3-3.5 $\mu$ .

Isolations from single conidia were effected by the dilution poured plate method. The course of germination is not different from many forms which have been described. There is at first a very considerable swelling followed, within 18 hours, by the formation of one or two terminal germ tubes. In the course of a few days, white; floccose colonies are formed. The portion of the mycelium in contact with the substratum blackens with age. In such media as 1 per cent dextrose plus 2 per cent agar, pycnidia are sparsely developed.

Seedling tobacco plants in flats in the greenhouse were atomized with a suspension of conidia from pure culture in tests to determine pathogenicity. Inoculations were made late in the afternoon and the flats were covered during the night with a sheet of paper in order to preserve a high relative humidity. Infections were evident within a week and within 7 to 10 days later, mature pycnidia were present within the invaded tissues. These were similar morphologically to those from which the original isolations were made.

It has been impossible to establish with certainty, the identity of this organism by comparison with published descriptions of the several species of *Phyllosticta* occurring on *Nicotiana tabacum*. It differs from *Phyllosticta tabaci* Pass., collected in Italy, principally in the appearance of the lesions and color and distribution of the pycnidia. It differs from *P. capsicola* Sacc. and Speg., also described from material collected in Italy, since the pycnidia of this species are grouped, the ostiole is somewhat beaked, and the conidia are curved and guttulate. *P. nicotianicola* Speg. on *Nicotiana acutiflora* from Argentine, differs in that it forms whitish leafspots, and its conidia are sub-cylindrical although its pycnidia with respect to distribution, size, shape and color are like those of the organism in question. It is manifestly very different from *Phoma nicotianae* Maubl. from France, and it is not believed that sufficient significance should be attached to the occasional occurrence of septate conidia to warrant regarding it as an *Ascochyta*.

Apparently the only known North American *Phyllosticta* on tobacco is *P. nicotiana* E. and E., from North Carolina, type specimens of which are missing from the Ellis and Everhart exsiccati in the herbarium of the New York Botanical Garden.<sup>1</sup> The meagreness of the original description<sup>2</sup> of this form gives little with which to make comparison

<sup>1</sup> Thanks are due to Dr. F. J. Seaver for his courtesy in examining the collections of the N. Y. Botanical Garden for me.

<sup>2</sup> Ellis, J. B., and Everhart, B. M. New species of North American fungi, Proc. Acad. Nat. Sci. Phila., p. 157, 1893.

with the material in hand. Whether or not they are identical can only be a matter of opinion. The discrepancy in the size of conidia, since those from Ellis and Everhart's type are described as about half that of my material, may be accounted for by an error in calibration or a shrinkage of conidia through desiccation before measurements were made. Since this cannot now be determined because of the loss of type material and since it does not appear that confusion would arise were one to reestablish the type on the basis of the material in hand, it is regarded as identical with *Phyllosticta nicotiana*. In order to preserve the writer's material, therefore, and make it available to students of tobacco diseases, it has been deposited in the herbarium of the Office of Pathological Collections of the Bureau of Plant Industry, Washington, D. C.

### BANANA FRECKLE IN THE PHILIPPINE ISLANDS

H. ATHERTON LEE

WITH ONE FIGURE IN THE TEXT

A black spotting is very common upon the banana fruits in the public markets in the Philippines. Both green and mature fruits show this disease. The spots are a dark reddish brown on certain green varieties but on others are black, minute, erumpent, and hard. Usually they occur in great numbers and in some cases in masses on the fruits. The spots upon the leaves are similar to those on the fruits but frequently run in streaks giving much the same appearance as melanose or tearstain on oranges. An affected leaf rubbed with the finger has a rough feeling suggestive of sandpaper. The Latundan, Borongan, Lacatan, and Saba (local dialect) varieties of *Musa sapientum* are affected.

Examination of the black spots shows the presence of pycnidia, which contain densely granular, oval, or irregularly shaped spores, having a thick hyaline envelope, and frequently a short hyaline appendage. The pycnidia and spores resemble very closely those of the fungus described as *Phoma musae*, Carpenter. Attempts to culture the organism have been unsuccessful, this experience being similar to that described by Carpenter<sup>1</sup> who mentions in his description that he was unable to grow it. The black spotting is entirely similar to the disease banana freckle as pointed out to the writer in the Hawaiian Islands by Professor Carpenter.

The disease is much more abundant at the close of the wet season than during the dry season. Since bananas in the Philippines are grown entirely for local consumption, the disease here does not cause the loss which occurs to the banana industry in the Hawaiian Islands.

<sup>1</sup> Carpenter, C. W. Banana freckle or black spot disease. Report of the Hawaii (Federal) Agric. Station, 1918: 36-40. Pl. 8-9. 1919.

A recent trip into the Sulu Archipelago and the Island of Mindanao has shown this disease to be widespread throughout those regions. In as much as these islands are but sparsely populated and importations of bananas from the western hemisphere have been unknown, the indications are that banana freckle is indigenous or at least of long duration here. In this connection it is of interest to quote from Professor Carpenter's report.

" . . . . . A year ago this disease appeared to be confined almost exclusively to the valley of Kalihi, (Territory of Hawaii) where it was serious but it could scarcely be found in Manoa Valley, 5 miles distant. It is now present to an alarming extent in the plantations of Moanalua, Pearl City, Mokuleia and Kahaluu, as well as in Kalihi and Manoa. During a recent trip to Kalihi Valley, it was impossible to find a bunch of fruit not affected . . . . ."

"Whether this is a new disease in Hawaii cannot be decided, though the writer inclines to the view."



Fig. 1. Freckle disease of bananas, similar to that described from Hawaii by Carpenter. On Latundan variety, from Manila markets.

The finding of banana freckle, so commonly and so widely distributed in the Philippines, would apparently corroborate Carpenter's view. There is a continual migration of labor from the Philippines to the sugar plantations of Hawaii and in view of this it is easy to understand the course by which a few affected fruits could reach the banana plantations of Kalihi Valley, adjacent to Honolulu.

BUREAU OF SCIENCE,  
MANILA, P. I.

**ABSTRACTS OF PAPERS PRESENTED AT THE FIFTH ANNUAL MEETING  
OF THE PACIFIC DIVISION, AMERICAN PHYTOPATHOLOGICAL  
SOCIETY, BERKELEY, CALIFORNIA, AUGUST 4 to 6, 1921.**

*Experiments on the use of oiled fruit wraps for the control of apple scald.* CHARLES BROOKS, J. S. COOLEY, AND D. F. FISHER.

Experiments conducted during the past year have confirmed results previously reported by the writers that scald can be successfully controlled under the most unfavorable storage conditions by wrapping apples in paper infiltrated with various oils which absorb the injurious respiration gases of the fruit. Oils heretofore used have tainted the fruit but during the past season an odorless and tasteless type of oil has been tested and has proved completely successful.

It was also found that apple scald could be arrested or reduced after several months storage by removing the common paper wraps and substituting oiled paper; likewise that if the fruit was first stored in oiled paper scald did not result when it was repacked in common paper after as short a time as one month.

*An outbreak of powdery mildew (*Podosphaera leucotricha*) on pears.* D. F. FISHER.

An outbreak of powdery mildew on pears occurred in the Central Washington fruit districts in the spring of 1921. Twig and foliage infection was not general but was confined to tender "water-sprouts" and terminals. The fruit, however, was very generally infected, orchards of D'Anjou variety commonly showing nearly every pear infected. Louise Bonne was nearly as susceptible as D'Anjou but Bartlett was considerably less so, while Flemish Beauty and Winter Nelis were markedly resistant. Conidia developed very freely and about July 1 perithecia began to appear as *Podosphaera leucotricha*, the same as on the apple. The disease has been epidemic on apples in this region since 1914 but has heretofore been noted only rarely on pears, although the trees are generally interplanted. The morphological characters of the fungus on pears do not differ from those found on apples, but the general occurrence of perithecia on the fruit instead of twigs is a noteworthy difference. The effect of the disease upon the fruit is the production of a black or russetted disfiguration, and in some cases a distortion of shape somewhat like early scab infection. There is also evidence that the disease caused an abnormal drop of fruit especially on D'Anjou.

*Notes on bacterial gummosis of stone fruits.* J. T. BARRETT.

The disease designated "bacterial gummosis" of stone fruits and caused by *Pseudomonas Cerasus* Griffin, has been under observation in California since 1916. Recognized at that time in only two rather widely separated counties it is now known to be widely distributed throughout the State. Attention was first directed to severe attacks on the apricot which in California seems to be very susceptible to the disease. There is, however, a great difference in severity on the apricot in different sections, and some evidence that there exists a difference in varietal susceptibility. In the northern part of the State the cherry is also attacked but not so severely as has been reported in the more humid sections of Oregon. In April of this year young peach buds in the nursery were attacked, many being killed, others badly injured. A difference in varietal resistance was apparent. In the same nursery and others, one very distant, young apricot

buds were similarly but more severely attacked. In one case 75 per cent of the new buds were killed in a three acre tract. Infection may take place from October to May but greatest activity on the apricot has been observed from January to April. In some of the old cankers the organism lives over the summer and together with spring infected fruit spurs furnish a source of inoculum for the following season. Preliminary tests indicate that from 20° C. to 27° C. is the range of temperature for optimum growth of the organism.

*Die-back of loganberry in the Northwest.* S. M. ZELLER.

Die-back of the canes of loganberry is very prevalent in the Northwest this year, especially where the canes were left down during winter. In yards having the canes trained up in the fall no die-back is manifest. *Mycosphaerella rubina* has been identified with some of the die-back but usually as an affection subsequent to some other causal condition. Low temperatures sufficient to injure canes have not been experienced and the root systems are usually in normal vigor. Evidence points to the conclusion that the canes are devitalized by the extreme moist conditions to which they are subjected during the winter rather than to the effects of any parasitic organism or low temperatures.

*Effect of alkaline sprays on the size of sweet cherries.* D. F. FISHER.

During spraying experiments for the control of brown rot of sweet cherries at Salem, Oregon, in 1915, 1916, and 1917, it was observed that a reduction in size of the fruit resulted from the application of fungicides. The repeated heavy application of self-boiled lime-sulphur 8-8-50 and Bordeaux 2-4-50, renders the fruit unmarketable through shrivelling, and the trees were not picked. The dwarfing was not as noticeable with commercial lime-sulphur 1-50. An explanation of this dwarfing was sought in special experiments in which various fungicides and components of the same were applied to different varieties both at Salem, Oregon, and Wenatchee, Washington. Check or unsprayed areas were retained on each tree to give comparable fruit and to eliminate variations due to individuality of the trees. Two applications were made, one as soon as the cherries began to color and another about two weeks later. In each case reduction in size of the ripe cherries was in proportion to the amount of alkaline material in the spray. A lime and lampblack wash resulted in no greater dwarfing than occurred from the application of lime alone, indicating that reduction of illumination was not the cause of the smaller fruit. The results indicate that dwarfing by alkaline sprays is brought about through excessive transpiration or water loss occasioned by the destruction of the waxy bloom.

*The parasitism of bastard toad flax (Comandra pallida A. DC.).* D. F. FISHER.

A study has been made of the parasitism of bastard toad flax in the region about Wenatchee, Washington, where this plant, *Comandra pallida*, is commonly found among the native vegetation of sagebrush lands. It was first observed growing under apple trees in a neglected orchard in 1919, but its parasitism on apple roots was not established until a year later when a more detailed survey and study was made. At that time specimens were sent to Dr. C. V. Piper and were identified by him as *Comandra pallida*. This parasitic attack on apple roots was described by Piper before the Botanical Society of Washington. *Comandra* has since been found parasitic on roots of the peach but this host was less affected than the apple. Pear trees and alfalfa growing in the same orchard were not attacked. Among the native plants the following were attacked; sagebrush (*Artemisia tridentata*), lupine (*Lupinus suksdorfii*), and yarrow (*Achillea millefolium*).

*European canker in Pacific Coast States.* S. M. ZELLER AND C. E. OWENS.

The European canker of apple (caused by *Nectria galligena* Bres.) has been known in Oregon since 1918, but not until after the extreme low temperatures experienced in December, 1919, was its wide distribution noted. At present the hosts for this canker, upon which the *Nectria* or *Fusarium* stages have been definitely identified in Oregon are several varieties of apples, namely; Red Cheek Pippin, Bismark, Delicious, Bell-flower, Ortley, Spitzenburg and Newtown; D'Anjou, Howell, and Bosc pears; *Quercus garyana*, *Acer macrophyllum*, *A. circinatum*, *Cornus Nuttallii* and *Salix* sp. It is reported from Humboldt Company, California, on apple. It is extremely virulent on Bosc and D'Anjou pears, producing cankers 20-22 inches in length in one season.

*Occurrence of Tylenchus dipsaci on alfalfa in Oregon.* M. B. MCKAY.

Specimens of diseased alfalfa plants sent to our laboratory from one farm under irrigation near Hermiston, Oregon, in June, 1921, showed the presence of and typical injury from the leaf and stem-infesting nematode, *Tylenchus dipsaci* (Kühn) Bastian. This pest has apparently not been previously reported on alfalfa in America though it has long been known on this crop in Europe and southern Africa where it has caused serious losses. The infested stems sent were usually much shortened and noticeably swollen and the epidermis over the infected areas was considerably wrinkled and corrugated. Some of the stems were killed entirely. As a consequence the stand was appreciably thinned and the first cutting of hay was light. It is not known how long the pest has been present on alfalfa in the region though similar injury was noted by the grower last year. The nematode from alfalfa was readily transferred by inoculations to clover on which it is causing typical injury. This nematode occurs as an economic pest also on clover and strawberry in Oregon and has been found causing damage in both irrigated and non-irrigated regions.

*Minimum incubation periods of causative agent of curly leaf in beet leafhopper (Eutettix tenella Baker) and sugar beet.* HENRY H. P. SEVERIN.

The beet leafhopper is non-virulent when it hatches from the egg. Curly leaf is not transmitted through seeds from "stechlinge" affected with the disease before and after transplanting. The beet leafhopper is not a mechanical carrier of curly leaf, nor a mechanical carrier in mass infection of a beet. The minimum incubation period of the causative agent of curly leaf in the beet leafhopper required four hours at the following temperatures: maximum 103°; minimum 94°; and mean 100° F.; and five days in the sugar beet at the following temperatures: maximum 93.6°; minimum 53.3° and mean 73.5° F.

*Transit diseases: An important factor in the cost of fresh vegetables.* GEO. K. K. LINK.

*Curly top of beet. Is it a mosaic disease?* EUBANKS CARNSNER.

*A Phomopsis from the Isle of Pines.* W. T. HORNE.

Grape fruit from the Isle of Pines, W. I., were secured from Mr. Frederick Maskew, State Commissioner of Horticulture, Quarantine Division, San Francisco, in October 1917, with stem end rot. From one of these fruits a fungus of the general *Phomopsis* character was isolated, which was capable of producing typical stem end rot in oranges and grape fruit and developed readily in ordinary culture media. In green citrus twigs in culture tubes it developed readily and formed pycnidia copiously. The fungus does not correspond exactly in culture characters with *Phomopsis citri*, Fawcett, from Florida, being apparently more vigorous.



*Some notes on two cars of grapefruit from the Isle of Pines.* W. T. HORNE.

Two cars of grape fruit were received in San Francisco in October 1917. A number of these fruits were examined and the more common blemishes were noted and their significance for the California citrus industry discussed. These included rust mite injury, tear stain, melanose (?), scab, black thrips rings, storage spot, gum spot, *Diplodia* stem end rot, *Phomopsis* stem end rot, *Myriangium* sp., *Sphaerostilbe cocophila* Tulasne.

*Pathogenicity of the olive knot organism on hosts related to the olive.* C. O. SMITH.

This organism, *Pseudomonas savastanoi*, has been studied by other investigators and artificial inoculation on hosts other than the olive were always either doubtful or negative. The inoculations of hosts closely related to the olive was made during 1919 to 1921. The following hosts when inoculated gave knots or galls that closely resembled those on the olive: *Fraxinus velutina*, *Fraxinus floribunda*, *Adelia acuminata*. Other hosts reacted differently and definite lesions 5 to 15 mm. in size, having hypertrophies at the margin of wound, were developed. At the margin small galls 2 to 3 mm. in diameter often formed. Hosts reacting as described are *Chionanthus virginica*, *Osmanthus aquifolia*. Hosts having small, point-like growths which on further experimentation would probably give positive results, are privet, *Ligustrum ovalifolium* and *Jasminum primulinum*. Negative results on *Osmanthus fragrans*, *Vinea theretia nerifolia*, *Nerium oleander*, *Carissa grandiflora*, *Chrysanthemum frutescens*, *Elaeagnus angustifolia*, lilac, various species of prunus, and *Coprosma baucarii*. Positive results only obtained on hosts closely related botanically to the olive.

*Some studies relating to infection of and resistance to walnut blight, Pseudomonas juglandis.*  
C. O. SMITH.

The leaf and catkin buds situated near blight lesions may have the blight organism on their surface before new growth starts, as shown by dilution plates. Artificial inoculation on dormant buds by puncture, brushing, or atomizing, develops little or no infection on new growth. Buds advanced so as to show green leaf tissue may be readily inoculated. Catkins themselves become infected, showing black, watery areas. Pollen from such catkins when shaken into petri dishes with agar develop blight colonies. Soil has been tested as a carrier by inoculating and testing same by poured plate method, and by direct inoculation of nuts. Organism could not be cultured, or cause infection, after about nine days. Commercial varieties of English walnut artificially inoculated to test resistance gave Ehrhardt 27, Eureka 29, Placentia 34, and Seedlings 49 per cent.

*Internal decline of lemons.* E. T. BARTHOLOMEW.

This is not a report of completed work but merely a description of the disease. It is a physiological malady. It is often spoken of commercially as "Blossom end decay," "Tip deterioration," "Pink tip," etc. It is characterized by the breaking down and drying out of the internal tissues usually at the blossom end of the fruit. It is usually first noticeable in the inner layers of the white parenchymatous tissue of the rind. The cells become disintegrated and a brown to pink gummy substance takes their place. From here the disease rather rapidly progresses into the pulpy tissues below and they become dry and light brown in color. The trouble does not appear until the fruit is about ready to pick, but then it may be found in green, silver, or yellow fruits, usually in the two latter. The disease does not appear to progress in the fruits after they have been taken from the trees. This disease is of very great economic importance for it often makes from 40 to 50 per cent of a pick of no value except for the by-products laboratory.

*Acid and water content of lemon fruits at different stages of development.* E. T. BARTHOLOMEW.

To study the cause of "Internal Decline," young lemons were measured, tagged, and remeasured once every month until mature. Each month some fruits were brought to the laboratory and tests were made to determine relative percentages of acidity and water content in the ends of the fruits. The acidity was found to be approximately the same in both ends of the lemons at all stages. The acidity gradually increases from about pH 4.46 in fruits  $\frac{3}{4}$  inch in diameter to about pH 2.23 in mature fruits. Fruits remaining on the tree after maturity show a small decrease in acidity. The acidity of the abnormal tissue was found to be pH 0.1 less than that of normal tissue. The water content of the blossom end is usually slightly greater than that of the stem end. Both rind and pulp were included in determining the water content. The water content of lemons  $\frac{3}{4}$  inch in diameter is about 53 to 55 per cent; that of mature fruits about 88-90 per cent. Fruits set in the spring grow much more rapidly and mature much quicker than those set at any other time. It may take 7 to 12 or even 14 months for lemons to mature, depending upon the time of the year at which they set.

*A Phomopsis of Citrus in California.* H. S. FAWCETT.

A Phomopsis somewhat resembling *P. citri* in Florida, and first found by O. F. Burger in California lemon shipments to Philadelphia, has been studied. Up to the present time it has been found only in Santa Barbara and Los Angeles counties. This California Phomopsis in cultures has a somewhat different mycelial growth, sporulates more freely and has different temperature relations to that of the Ebrida Phomopsis. Its ability to enter and break down fruit has been shown by inoculation experiments to be very weak as compared to the Florida Phomopsis. It has been found in the packing house only on old overripe fruit, and in the orchards on a few dead twigs only after considerable search. Traces of what appeared to be mild melanose markings (a conspicuous result of *Phomopsis citri* attack in Florida) were also found on a few specimens of pomelo fruits in Santa Barbara County. The lack of virulence of this Phomopsis and, therefore, the minor importance of the effect produced by it probably accounts for its having been previously overlooked.

*The relation of Citrus blast to certain environmental factors.* H. S. FAWCETT.

The organism (*Bacterium citriputeale*), which has been shown to be responsible for both citrus blast and black pit is markedly sensitive to slight changes in climatic conditions. It is active only in the rainy season during a short period in late winter and early spring. The history of its occurrence since 1912 at Oroville studied in connection with weather data, shows that whenever the number of rainy or cloudy days falls below normal the disease is mild or unimportant and whenever the rainy or cloudy days are above normal the disease is severe. It has also been shown statistically that the disease has a direct relation to injuries to the foliage by winds when accompanied or followed by rains. The organism has a low optimum temperature for growth and infection and is dependent upon cool as well as moist weather condition and injuries for infection and development.

*Some fig diseases.* EDITH H. PHILLIPS.

1. Fig smut. An *Aspergillus* attacks the insides of the fruits as they begin to dry. This fungus has been found to carry over in small twigs.
2. *Sclerotinia libertiana* causes a die-back of fig twigs in the spring.
3. A *Botrytis* also causes a die-back. It enters the twigs through hang-on figs in the fall, or through frost-injured tissue in the spring.
4. An undetermined fungus causes a rot of fig fruits and subsequent cankers at the points where the rotted figs hang.

*Data relative to the germination of aeciospores, urediniospores and teliospores of Puccinia coronata Cda.* G. R. HOERNER.

Summary of results: Aeciospores from herbarium specimens of *Rhamnus* were not viable after a period of 167 days from date of collection. Urediniospores from herbarium specimens of *Avena sativa* L. proved to be viable as long as 87 days after date of collection. Unprotected, urediniospores lost their viability within 22 days with a minimum temperature during this period of  $-27^{\circ}$  F. and a maximum of  $42^{\circ}$  F. When afforded protection with a temperature range similar to the unprotected, these spores remained viable as long as 44 days. Exposed to light, viability of urediniospores was lost within 23 days, during which period the maximum temperature was  $86^{\circ}$  F. and the minimum  $29^{\circ}$  F. Kept in the dark, urediniospores at similar temperatures to those exposed to light, remained viable as long as 79 days. Urediniospores germinated at a temperature as low as  $7^{\circ}$  C. with an optimum of  $18^{\circ}$  C. and a maximum of  $32^{\circ}$  C. Teliospores developed on oat seedlings in the greenhouse and not afforded a period of overwintering, did not germinate. Previous to overwintering and as late in the spring as May 2, teliospores developed in the field were incapable of germination.

*Miscellaneous studies with crown rust of oats.* G. R. HOERNER.

Summary of results: Urediniospores borne on the surface of oats seed do not offer a ready means of infecting seedlings developed from these seeds. Seedlings of oats emerging through soil heavily covered with viable urediniospores are not readily infected. Under Minnesota conditions, a perennial mycelium capable of producing a new crop of urediniospores after overwintering, does not exist. What the situation is in the case of wild grasses has not been determined. Urediniospores do not remain viable over winter on oats, nor does continued production take place. What the situation is in regard to wild grasses has not been determined. Environmental factors influenced the development of the rust on oats as well as the rate of pustule formation. Etiolation brings about the early formation of telia on oat seedlings. Anthocyanin pigment formation surrounding uredinia on infected oat leaves is a common phenomenon though not correlated with resistance or susceptibility. The appearance of telia on seedling oat leaves is not a reliable basis for determining resistance of oat varieties.

*Observations on the nature of resistance to stem rust attack in certain resistant and susceptible wheats.* RUTH F. ALLEN.

*Resistance in oats to stem rust attack.* W. W. MACKIE AND RUTH F. ALLEN.

*Recent investigations on rice disease in the United States.* W. H. TISDALE.

*The present status of cereal pathology in the United States.* H. B. HUMPHREY.

*The use of chemical dusts to prevent bunt.* W. W. MACKIE AND FRED. N. BRIGGS.

*The relation of soil moisture and soil temperature to bunt infection in wheat.* C. W. HUNGERFORD.

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# PHYTOPATHOLOGY

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## TWO IMPORTANT PINE CONE RUSTS AND THEIR NEW CRONARTIAL STAGES

Part 1. *Cronartium strobilinum* (Arthur) Hedge. and Hahn, comb. nov.

GEORGE G. HEDGCOCK AND GLENN GARDNER HAHN

WITH PLATES V AND VI

The Florida pine cone rust hitherto known in the literature as *Caeoma strobilina* Arth. was described by Arthur<sup>1</sup> from diseased cones of *Pinus palustris* Mill., received from P. H. Rolfs, Palatka, Florida. In the aecial stage this fungus has been known in this state since 1892, when it was collected for the first time by Mr. W. T. Swingle. The distribution of the rust not only includes Florida, where it occurs abundantly in certain localities, but also the neighboring state of Mississippi.<sup>2</sup> At this writing a field survey is incomplete and there is no definite data to show exact distribution, and whether the rust is spreading in the southern states. Frequently the disease has a very damaging effect on the reproduction of the species of pines upon which it occurs. In May, 1919, a count was made of the diseased cones of *P. heterophylla* (Ell.) Sudworth (*P. caribaea* More.) and *P. palustris* over two acres at Dunedin, Florida, and the approximate number of infected cones per tree varied from 25 to 90 per cent. of the total cone crop. This particular year the disease was very common from Palatka and Lake Butler to Tampa. In certain localities where the rust occurs, reproduction of the two pine species common in Florida is very scant. This continuous destruction of the seed crop each year cannot fail to be a hindrance to reforestation; therefore the pine cone rust must be considered as a limited but very important disease.

<sup>1</sup> Arthur, J. C. New species of Uredinieae—V. Bull. Torrey Bot. Club **33**: 519–520. 1906.

<sup>2</sup> The senior writer noted the occurrences of what appeared to be the mature aecial stage of this pine cone rust at a number of points along the coastline of the A. C. L. R. R. in Georgia in May, 1919, but no collections were made. Also observations of persistent mummy cones were made March, 1915, at Dothan, Alabama.

## DESCRIPTION OF THE DISEASE

The immature first year cones become infected at an early stage in their development, and within two or three months after they emerge many often exceed the size of the second year uninfected cones (Pl. V, fig. A) borne on the same tree. So far observations do not include infection of these two year old healthy cones. During the first of March many infected scales bearing pycnia may be observed accompanied by immature aecia within the cortical layers of the cone scale tissue immediately beneath. With the maturity of the parasite, the infected cones have become abnormally enlarged and each individual swollen scale has a reddish color which is at first an orange chrome, becoming later a Sanford's brown or chestnut.<sup>1</sup> With the rupturing of the aecial cavities and the sloughing off of the diseased cone scale tissue above, powdery masses of cadmium yellow spores are laid bare, (Pl. V, fig. B,) which entirely cover the cone. Excessive hypertrophy results, and the cones present a large swollen mass or gall, externally still showing the identity of each individual scale, but in longi-section, showing partial fusion of the scales.

In the spring the presence of the young infected cones bearing pycnia can be detected in the tops of tall trees by the presence of nectar-loving insects which have been observed to feed on the pycnial exudations. Later the diseased cones are attacked by insects. One of these which is commonly associated with infected cones is *Dioryctria abietella* D. & S.<sup>2</sup> By autumn the diseased cones have all died and most of them fallen to the ground. Here and there a mummy cone remains indicating the presence of the disease.

## ARTIFICIAL INOCULATIONS

Search for the alternate stage of the so-called *Caeoma strobilina* was made by Hedgcock for three successive seasons. The only rust found commonly associated with the Caeoma in pine cones was a *Cronartium* which wintered over on the evergreen oaks. In a previous publication<sup>3</sup> this overwintering habit was attributed to *Cronartium cerebrum* (Peck) Hedgc. and Long. In view of our present knowledge of the rust, we now know that this overwintering fungus was largely the cone rust fungus. As to the exact amount of overwintering of *C. cerebrum*, the writers are not prepared to state at this time.

<sup>1</sup> Colors used are those of Ridgway, R., Color Standards and Nomenclature, Washington, D. C., 1912.

<sup>2</sup> Identification made by Dr. A. D. Hopkins, Bureau of Entomology.

<sup>3</sup> Rhoads, A. S., Hedgcock, G. G., Bethel, E., and Hartley, C. Host relationships of the North American rusts, other than the Gymnosporangiums, which attack conifers. *Phytopathology* 8: 315-316. 1918.

In connection with the above mentioned field observations artificial inoculations were made in the Pathological Greenhouses, Washington, D. C. Inoculations with a given strain of the fungus from a given host and locality were made upon healthy, uninfected plants with all aseptic precautions. These plants were then isolated in separate greenhouses or separate compartments made of cheesecloth upon the side benches of the greenhouse. In no instance were strains of the rust ever mixed. In making observations a single strain was examined upon a given day. All precautions were taken to guard against any possible contamination of an isolated strain.

On March 26, 1918, fresh aeciospores of *Caecoma strobilina* from cones of *Pinus heterophylla* collected at Dunedin, Florida, March 20, were used in inoculating the leaves of two oak trees, *Quercus gambelii* Nutt. and *Q. prinus* Linn. in a moist chamber. On April 11, numerous mature uredinia of a *Cronartium*<sup>1</sup> appeared on the leaves of both trees. A second inoculation with the same aeciospore material mentioned above was made April 4 on the leaves of a number of oak trees, and on May 18 abundant mature uredinia of a *Cronartium* were present on the following trees: 2 *Quercus coccinea* Muenchh., 5 *Q. gambelii*, 1 *Q. phellos* Linn., 1 *Q. prinus* and 1 *Q. virginiana* Mill. A third inoculation was made on oak trees May 14 with fresh aeciospores sent from Dunedin, and June 1 the following trees were infected, bearing mature uredinia: 4 *Quercus coccinea*, 2 *Q. emoryi* Torr., 4 *Q. gambelii*, 1 *Q. michauxii* Nutt., 3 *Q. minor* (Marsh) Sargent, 1 *Q. palustris* Muenchh., 1 *Q. phellos*, 4 *Q. platanoides* (Lam.) Sudworth, 1 *Q. rubra* Linn., 3 *Q. velutina* Lam. and 2 *Q. virginiana*.

Pedigreed urediniospores from the preceding experiments were used to propagate and continue the growth of the rust on oak trees for the remainder of 1918 and in 1919. Only one of the trees originally inoculated, a tree of *Quercus virginiana*, which did not shed its leaves, carried the rust through the winter of 1918-1919. Two leaves of this tree bore a few telia of a *Cronartium* which were mature about December 15, 1918.

In 1919 fresh aeciospores were obtained from *Pinus heterophylla* and *P. palustris* at Dunedin,<sup>2</sup> Florida, and from these strains inoculations were made independently and apart from those of the previous year's rust strains. The results obtained were similar to those of the previous year. With these pedigreed strains from 1918-1919 aeciospores, trees of the following additional species in 1919 were infected successfully, bearing urediniospores: 1 *Quercus alba* Linn., 4 *Q. dentata* (Marsh) Borkh.,

<sup>1</sup> These, owing to the inconspicuous peridium were at first assumed to belong elsewhere than to *Cronartium*. See *Phytopathology* 8: 338, 1918.

<sup>2</sup> No stem Peridermium of any species was found present in or near Dunedin during a survey of three years.

*Q. geminata*, 2 *Q. gunnisonii* (Torr.) Rydb., and 10 *Q. robur* Linn. From the pedigreed 1918 strains the following bore the telia of *Cronartium*: 1 *Quercus robur*, and 5 *Q. virginiana*, from the 1919 strains, 1 *Q. minor*, 1 *Q. rubra*, and 5 *Q. virginiana*.

In 1920 and 1921, further aecial and uredinial inoculations were made confirming the work of the preceding years, many dark telia of *Cronartium* being formed on the leaves of the infected trees from December, 1920, to March, 1921. No telia were formed in any of the experiments during the period from April to November.

During four years' experimentation the number of trees of each species successfully infected and bearing uredinia are as follows, the number in parenthesis indicating the number of trees bearing telia: 16 *Castanea dentata* (Marsh) Borkh., 5 *C. pumila* (Linn.) Mill., 5 *C. sativa* Mill., 36 *Quercus agrifolia* Née, 9 *Q. alba* Linn., 1 *Q. californica* (Torr.) Coop., 6 *Q. cerris* Linn., 12 *Q. coccinea* (2), 27 *Q. dentata*, 18 *Q. emoryi* (4), 18 *Q. digitata* (Marsh) Sudworth, 7 *Q. douglasii* Hook & Arm., 50 *Q. gambelii*, 11 *Q. geminata* (3), 13 *Q. gunnisonii*, 6 *Q. imbricaria* Michx., 3 *Q. lobata* Née, 5 *Q. macrocarpa* Michx., 5 *Q. michauxii*, 6, *Q. minor* (1), 5 *Q. palustris*, 3 *Q. phellos*, 7 *Q. platanoidea*, 1 *Q. prinoides* Willd., 16 *Q. prinus*, (1), 14 *Q. robur* (4), 12 *Q. velutina*, and 37 *Q. virginiana* (17). We have successfully infected with this rust every species of *Castanea* that we have tested. *Quercus virginiana* and *Q. geminata* in the greenhouse as well as in nature are the best telial hosts.

Inoculations with fresh aeciospores collected from *Pinus heterophylla* Dunedin, Florida, were made March 26, 1918, as follows: 4 first year cones of 2 *P. pinaster* Ait. were inoculated by inserting aeciospores in wounds beneath the epidermis, and closely wrapping with moist cotton; 6 first year cones were inoculated by placing aeciospores upon the surface of the cones, and then similarly wrapping with moist cotton. Negative results were obtained in both cases.

Inoculations with teliospores were made in a number of pine trees in 1919 and 1921, by placing telial columns in incisions under the bark of rapid growing shoots, and tightly wrapping the wounds with moist cotton. Negative results have been obtained to date. Trees as follows were used: 9 *Pinus heterophylla*, 1 *P. clausa* (Engelm.) Sarg., 30 *P. radiata* Don., and 2 *P. taeda* Linn. Inoculations similarly made in other experiments with the telia of *Cronartium cerebrum* (Peck) Hedge. and Long, and *C. fusiforme* (Peck) Hedge. and Hunt, have given a high percentage of infection both for 1919 and 1921 inoculations.

The above investigations made by the writers<sup>1</sup> 1915-1921 now clearly

<sup>1</sup> N. Rex Hunt should be credited with part of the earlier studies and culture work at Washington, D. C., in 1918.

show that *Caeoma strobilina* is a true Peridermium with definite peridia belonging to a Cronartium occurring on deciduous and evergreen oaks. We therefore transfer *Caeoma strobilina* Arth. to the genus Cronartium with the following description:

CRONARTIUM STROBILINUM (Arthur) Hedgec. and Hahn, comb. nov.

Syn. *Caeoma strobilina* Arthur, Bull. Torr. Bot. Club **33**: 519, 520, 1906.

O. Pycnia<sup>1</sup> conicolous, preceding the aecia the same season,<sup>2</sup> arising in the infected reddened cone scales in sub-epidermal ellipsoid areas which finally coalesce into a more or less broken layer, 90 to 380  $\mu$  deep, averaging 200  $\mu$ ; pycnial exudate deep chrome to yellow ochre; pycniospores obovoid to ellipsoid, hyalin, 2 to 2.5  $\mu$  by 2.5–3.5  $\mu$ , averaging 2.2 by 3  $\mu$ .<sup>3</sup>

I. Aecia conicolous, forming in hypertrophied scales, in deep seated sub-corticular ellipsoidal areas (Pl. V, fig. C) several layers of cells (6 to 14) beneath the pycnial layer which finally coalesce into a more or less broken layer within the cortex of the cone-scales; peridium in a definite multiple layer; peridial cells<sup>4</sup> (Pl. V, fig. D) at the top of the sorus. are usually more rounded than those at the sides; for the latter tend to become pulled out or elongated. Therefore the size of the cells varies widely, 10–20 by 10–45  $\mu$ . The mature wall is 3–5  $\mu$  thick, averaging 4  $\mu$ ; outermost wall being smooth and inner cell wall verrucose with short tubercles which in clumps tend to give the cells the appearance of meshing or dove-tailing together. The protoplasmic contents of the peridial cells stain very lightly as compared with the aeciospores. Fre-

<sup>1</sup> The pycnia are described from F. P. No. 25179 *Pinus heterophylla*, Dunedin, Fla., March 20, 1918; and the aecia from F. P. No. 22747 from *Pinus palustris*, Fruitland Park, Fla., May 8, 1918.

<sup>2</sup> The pycnia of this species are unique because they differ from other known species of Cronartium, in that they are formed the same season in the same tissues as the aecia but mature earlier. Pycnia formed a short time before the aecia which lie beneath are separated from the latter by several layers of cortical cells.

<sup>3</sup> The size of sori in this description are based on not less than 50 measurements, and of spores on not less than 100 measurements. \*

<sup>4</sup> The peridium of *Cronartium strobilinum* in the aecial stage is unique with regard to its formation. Due to the deep seated origin of the aecial area, the peridial layer never becomes extruded from the cone scale (Plate V, fig. C) tissue, but always remains more or less deeply buried beneath the cortical layers above. With the sloughing off of these cortical layers above the aecium, the peridium falls away at the same time with these outer tissues, leaving the base of the aecium apparently without a peridium. Sections of the immature rusted cones readily show this peridium, the cells of which can be readily identified as peridial cells by their position, shape, and cellular content.



quently these cells are merely empty shells. Fresh aeciospores in mass are cadmium yellow to capucine yellow; aeciospores obovate to ellipsoid, rarely globoid, often attenuate below, 12–20 by 17–31  $\mu$ , averaging 15 by 25  $\mu$ , walls 3–5  $\mu$  thick, averaging 3  $\mu$ , verrucose with small tubercles 1.5 to 2  $\mu$  in diameter and with a smooth area, extending from the thickened base up one side.

II. Uredinia<sup>1</sup> hypophyllous, rarely epiphyllous, subepidermal, sometimes internal, at first from a limited mycelium, but later spreading; infected areas become brightly colored, sal non orange to grenadine red; sori produced continually throughout the year, both in the field and in the greenhouse, wintering over upon the leaves of evergreen oaks; sori sphaeroid to ellipsoid, rupturing at the top of the dome. Peridia delicate, evanescent; formed by a layer of cells which adheres to neighboring homologous cells (Pl. V, fig. E) and which gradually separates from the underlying urediniospores (Pl. V, fig. F). With the maturity of the urediniospores, the peridium is forced up into the dome, resulting in the loss of outline and regular shape of the peridial cells which become exceedingly flattened, and almost indistinguishable (Pl. V, fig. G) when the sorus is ruptured. Urediniospores, cadmium yellow in mass, globoid to ovoid or ellipsoid, 12–18 by 15–26  $\mu$ , averaging 15 by 20  $\mu$ ; walls colorless, 1.5–3  $\mu$  thick, averaging 2  $\mu$ , echinulate with low, inconspicuous, conical papillae, 1.5–2  $\mu$  in diameter.

III. Telial columns hypophyllous rarely epiphyllous, filiform, mummy brown to black, 1.5–3.8 mm. in length, averaging 2.6 mm., 70 to 190  $\mu$  in diameter, averaging 110  $\mu$ ; teliospores fusiform-oblong to fusiform, 10–20 by 20–45  $\mu$  averaging 15 by 30  $\mu$ , walls 3–6  $\mu$  averaging 4  $\mu$ , smooth, brownish.

#### COLLECTIONS

*Cronartium strobilinum* according to our records has been collected as follows:

I. On *Pinus heterophylla*: Florida—Altoona, by W. T. Swingle, May 31, 1892<sup>2</sup>; Orange Bend, June 4, 1892; Dunedin, S. M. Tracy,<sup>3</sup> June 20,

<sup>1</sup> The uredinia are described from type specimen F. P. No. 32454, and the telial columns from type specimen F. P. No. 38015, *Quercus virginiana*, Pathological Greenhouses, Wash., D. C., grown from aeciospores under controlled conditions. The type specimens are deposited in the Pathological Collections, U. S. Dept. of Agr., Wash., D. C.

<sup>2</sup> Pathological Collections, U. S. Dept. of Agr.

<sup>3</sup> Arthur, J. C., New Species of Uredineae V, Bull. Torr. Bot. Club 33: 519, 1906. Through the courtesy of Prof. Arthur, the writers have been permitted to examine and compare the specimens cited in this paper.

1921, (wrongly determined as *Pinus taeda*), G. G. Hedgcock, March 20, and May 8, 1918, March 5, and May 17, 1919; Lake City, by P. H. Rolfs,<sup>2</sup> May 30 and July 10, 1906 (wrongly determined as *Pinus taeda*); Florence Villa, by S. F. Poole, April 25, 1913; Eustis, by W. H. Savage, June 1, 1914, and by G. G. Hedgcock March 25, 1918, also Leesburg, March 8 and 26, 1918; Tampa, March 7, 1919; Gainesville, May 14, 1919. Mississippi—Agricultural College, by J. M. Beal, May 10, 1915; Hattiesburg, by J. W. Champlin, May 15, 1919.

On *Pinus palustris*: Florida—Altoona, by W. T. Swingle, June 1, 1892; East Palatka by P. H. Rolfs, May 30, 1906; Gainesville, by H. E. Stevens, May 23, 1914, and by G. G. Hedgcock, May 14, 1919; Fruitland Park, by L. P. Bosanquet, June 19 and 30, 1916, and June 1, 1918, and by G. G. Hedgcock, March 3, 1918; also Dunedin, March 20 and May 8, 1918, March 5, and May 17, 1919; Tampa, March 21, 1918, and March 7, 1919; Leesburg, March 26, and May 7, 1918, and May 8, 1919; Weirsdale, March 10, 1919; and Brooksville, May 16, 1919; Alton, by T. S. Tramel, May 12, 1921.

*Cronartium strobilinum* so far as is known at present has its aecial stage on the cones of only two species of pine, *Pinus heterophylla* and *P. palustris*.

II, III. On *Q. geminata*: Florida—by G. G. Hedgcock, West St. Augustine, March 4, 1915; Sopchoppy, April 3, 1915; Worthington Springs, March 29, 1918; Lake City, March 30 and 31, 1918 and March, 12, 1919; Gainesville, February 25, and May 14, 1919; Silver Springs, February 27 and May 15, 1919; Ocala, February 28, 1919; Brooksville, March 4, and May 16, 1919; Tampa, March 7, 1919; Leesburg, March 8, 1919; Jasper, March 13, 1919; Dunedin, March 5, and May 17, 1919, also by W. H. Long, January 21, 1919.

On *Quercus virginiana*: Florida—by G. G. Hedgcock, Brooksville, March 3 and 4, 1919; Weirsdale, March 10, 1919; Gainesville, May 15, 1919; Dunedin, March 5, 1919; also by W. H. Long, January 21, 1919. Cuba—San Diego de Los Baños, by J. R. Johnston and C. H. Ballou, March 25, 1921.

On *Quercus nigra*: Florida—Gainesville, by G. G. Hedgcock, February 25, 1919.

Specimens have been also collected from artificial inoculations at Washington, D. C., on nearly all the species of chestnut and oak used in the experiments given in the preceding pages of this paper.

In Florida, either *Quercus geminata* or *Quercus virginiana*, or both are always found in close association with pines infected with the cone rust. From this it is predicted that the range of *Cronartium strobilinum* will be limited to the natural range of these two telial hosts, since infected deciduous hosts shed their leaves before telial formation.

In the greenhouse at Washington, D. C., leaves of deciduous species of *Castanea* and *Quercus*, inoculated during the period from March to June, fail to produce telia because they shed their leaves normally before the time of telial production takes place. By cutting back trees and forcing them to renew their foliage so that they can be inoculated from July to December, telial formation is secured during the winter months. In evergreen species, cutting back is not necessary to secure telial production. The maximum period of telial formation in the greenhouse is during the months of December and January. Although observations have not been made, this period must coincide with what takes place in the field in order that the young cones may become infected at this time or shortly afterwards, with the result that pycnia are produced the latter part of February and the first of March.

Part II. *Cronartium conigenum* (Pat.) Hedge. and Hunt, comb. nov.

GEORGE G. HEDGCOCK AND N. REX HUNT

Patouillard<sup>1</sup> in 1896 described the new species *Caeoma conigenum* on the hypertrophied cones of an undetermined species of pine from a specimen collected by Maury in Mexico, June, 1891. He published a picture of a diseased and a healthy cone of what is undoubtedly *Pinus chihuahuana* Engelm. The photograph from which the plate was prepared was taken by Maury and sent with the type specimen to Patouillard in France. Although Patouillard describes a *Caeoma*, a *Peridermium* with cerebroid aecia is shown in figure 2 of his plate. The type specimen was sent a long distance and may have lost evidences of the peridium in transit. Through the courtesy of Prof. J. C. Arthur and of Dr. F. J. Seaver, the writers have examined two fragments of the type. The specimen from Prof. Arthur's collection shows an aecial layer and no distinct peridium but peridial cells were found among the aeciospores. The piece of the specimen sent by Dr. Seaver from the collections of the New York Botanical Garden also possessed an aecial layer and distinct traces of a peridium, proving that *Caeoma conigenum* Pat. is morphologically a *Peridermium*.

In recent years a number of collections of a pine-cone rust have been received by the writers from Arizona. The earlier collections were badly weathered, did not show a distinct peridium, and were assigned to *Caeoma conigenum*.<sup>2</sup> Later specimens possessed aecia with distinct

<sup>1</sup> Patouillard, N. Note sur un cone de pin déformé par une urédinée. Jour. de Botanique 10: 386-388, Pl. IV. 1896.

<sup>2</sup> Hedgecock, Geo. G. Notes on some western Uredinia which attack forest trees. Mycologia 4: 146, 1912.

erumpent, occasionally cerebroid peridia.<sup>1</sup> House receiving one of the specimens with cerebroid aecia identified it as *Peridermium cerebrum* Peck<sup>2</sup> and published a very fine illustration of it.

The junior writer in an investigation of this cone *Peridermium* in the vicinity of Portal, Arizona, in May, 1918, found both the fresh uredinial and mature telial stages of a species of *Cronartium* apparently wintering over very sparsely on the leaves of the evergreen oaks *Quercus emoryi* Torr. and *Q. hypoleuca* Engel., and occurring even more abundantly on the fallen leaves of the same species, and closely associated with trees of *Pinus chihuahuana*. The latter bore weathered cone-galls which had evidently fruited the previous year, since there were present abundant remnants of aecia, giving them a grayish color. The uredinia and telia were found at least two months before the time of maturity for the aecia on the cone-galls, which is July to August. No stem form of *Peridermium* was found in 1918 on the pines present in this region (*Pinus chihuahuana*, *P. apachea* Lemm., and *P. cembroides* Zucc.), although hundreds of trees were closely examined, nor has any other species of *Peridermium* ever been to our knowledge reported from this region. This *Peridermium* evidently infects the cones of *Pinus chihuahuana* during the first year of their growth. As a result of infection, cones develop into galls having a slight differentiation of the surface into scales and very little resemblance to a true cone. The immature galls are usually two or more times as large as cones of the same age (Pl. VI, figs. A and B) and regardless of size may be sliced up with a knife much more readily than healthy cones. After fruiting the galls dry up and may remain on the trees as mummies. The interior of these mummies is almost always nearly destroyed by insects, so that little more than a brittle shell remains after a couple of months. A striking feature of the disease is the great variation in the size of the galls, the largest having a volume ten times that of the smallest. Most of the galls are of the smallest size and evidently fruit within two years after infection, as shown by the age of the nodes bearing them, while the medium sized galls fruit (at least in part) within three years. The age, at the time of fruiting, of the largest galls was not determined, partly because insects usually kill them before they fruit and partly because they had invariably stopped the normal elongation of the branches bearing them. In fact such galls, even though not yet mature, almost always terminate the branches bearing them. Before fruiting, these largest galls have a much

<sup>1</sup> Hedgecock, Geo. G. and Hunt, N. Rex. Notes on some Uredinales attacking pines. *Phytopathology* 9: 53, 1919.

<sup>2</sup> House, H. D. *Peridermium cerebrum* Peck. Rept. New York State Botanist or 1914. *Museum Bull.* 179: 36, 37. With plate. 1915.

smoother surface than the smallest ones. Remains of the aecia are very evident on some cones for a year or more after sporulation. The finding of galls with the remnants of what appeared to be the aecia of two different years led to the unconfirmed suspicion that some galls might fruit for more than one season.

The economic importance of this cone disease cannot be determined without considerable study. Counts of diseased and healthy cones on several groups of trees showed that more than fifty per cent of the cones were infected in some groups, while on many individual trees as high as ninety per cent were infected. In addition to the diminution of seed production, the disease stunts or terminates the growth of many limbs so that infected trees are apt to present a very ragged appearance. Although only one small region has been surveyed, it seems quite possible that under favorable conditions this *Peridermium* might cause a dangerous disease, especially in northern Mexico where *Pinus chihuahuana* is of greater commercial importance.

#### ARTIFICIAL INOCULATIONS

The senior writer<sup>1</sup> has made inoculations with the aeciospores of this pine cone rust on *Pinus chihuahuana* from Arizona in greenhouses at Washington, D. C., as follows:

Immature aeciospores from unopened aecia on cones collected in the Chiricahua Mountains by A. H. Zachau of the Forest Service June 11, 1914, were inoculated without infection on 8 oak trees, viz., 2 *Quercus coccinea* Muenchh., and one each of *Q. arizonica* Sarg., *Q. emoryi*, *Q. michauxii* Nutt., *Q. minor* (Marsh) Sarg., *Q. palustris* Muenchh., and *Q. virginiana* Mill., without any infection. July 9, 1914, aeciospores from the same source were used to inoculate 5 trees of *Pinus virginiana* Mill., and 1 *P. rigida* Mill., by inserting masses of spores under the bark of growing shoots and wrapping the wounds closely with wet cotton, also without infection.

July 30, 1918, leaves of 39 oak trees were inoculated with fresh aeciospores collected by A. J. Abbott of the Forest Service near Portal, Arizona, July 26. Of these 6 trees became infected and bore the immature uredinia of a *Cronartium* in 10 to 12 days. One each of the following species were infected: *Quercus palustris*, *Q. phellos* Linn., *Q. prinus* Linn., *Q. rubra* Linn., *Q. velutina* Lam., and *Q. virginiana*. Some of the uredinia remained on the leaves until November, but owing to the shedding of the leaves no telia were formed.

<sup>1</sup> During 1920 and 1921 he was assisted by Glenn G. Hahn.

July 20, and July 30, 1920, leaves of 81 oak trees were inoculated with aeciospores from cones collected by Otto Schoenberg of the Forest Service near Portal July 14, 1920. The following became infected, bearing the mature uredinia of a *Cronartium* in 10 to 15 days: 2 *Quercus agrifolia* Née, 1 *Q. coccinea*, 4 *Q. douglasii* Hook & Arn., 2 *Q. gambelii* Nutt., 1 *Q. geminata*, 5 *Q. robur* Linn., and 1 *Q. rubra*. In this experiment the following chestnut and chinquapin trees were inoculated: 3 *Castanea dentata* (Marsh) Bork., and 1 *C. pumila* (Linn.) Mill. The leaves of the tree of *Q. geminata* had chlorotic spots indicating infection, but no uredinia were produced. At the same time the following pines were inoculated in the same manner as those in the experiment in 1914: 4 *Pinus heterophylla* (Ell.) Sudw. (*P. caribaea* More.), 4 *P. radiata* Don. and 2 *P. taeda* Linn. These are as yet without an apparent infection.

During 1920 and 1921, by using urediniospores from the preceding set of inoculations, many trees of oak, chinquapin and chestnut were inoculated, a number of which bore the telia of a *Cronartium* during the winter months from November to February, thus fully proving that this pine cone Peridermium belongs to the genus *Cronartium*. The following list gives the number of trees of each species successfully inoculated and bearing uredinia, the number bearing telia given in parentheses: 11 *Castanea dentata*, 9 *C. pumila* (1), 5 *C. sativa* Mill., 16 *Quercus agrifolia*, 9 *Q. alba*, 3 *Q. coccinea*, 15 *Q. cerris* Linn., 6 *Q. douglasii* (2), 6 *Q. gambelii*, 1 *Q. imbricaria* Michx., 14 *Q. macrocarpa* Michx., 1 *Q. marilandica* Muenchh., 5 *Q. minor* (Marsh) Sarg., 7 *Q. palustris* (1) 1 *Q. platanoides* (Lam.) Sudw., 9 *Q. prinus*, 32 *Q. robur* (6), 7 *Q. rubra*, 5 *Q. velutina* (1) and 1 *Q. virginiana*. An equal number of trees were used as controls in the experiments given in this paper, and all remained free from the rust. Trees when inoculated were placed for a period of 2 to 3 days either in enclosed glass walled damp chambers or in iceless refrigerators,<sup>1</sup> the latter giving the best results. After inoculation, the trees were kept in rooms or compartments of the greenhouses separate from trees inoculated with other species of *Cronartium*.

We consider *Caeoma conigenum* identical with this Arizona pine cone rust for the following reasons: Patouillard's illustration of a diseased cone agrees with the appearance of weathered cones diseased with the Arizona rust in which the aecia have broken through the outer layers of the cone tissues. His measurements for the aeciospores, 12-20 by 25-40  $\mu$ , and our measurements (based on 100) of the same from two

<sup>1</sup> Hunt, N. Rex. The "iceless refrigerator" as an inoculation chamber. Phytopathology 9: 211-212. May, 1919.

fragmental type specimens, 14–21 by 24–50 and 12–21 by 18–41  $\mu$ , fall well within the range obtained by us for those of the Arizona rust, viz., 12–25 by 19–51  $\mu$ . The peridial cells found in the type specimens agree closely in size and shape with those present in the Arizona rust. We now transfer *Caeoma conigenum* to the genus *Cronartium* with the following description:

*CRONARTIUM CONIGENUM* (Pat.) Hedgec. and Hunt comb. nov.

Syn. *Caeoma conigenum* Pat., Jour. de Bot. 10: 386–388, Pl. IV, 1896.

O. Pycnia<sup>1</sup> in lenticular, subepidermal areas, either preceding the aecia by one growing season, or occurring on different areas of the same cone scales later in the same season as the aecia. Pycnosporos obovoid.

I. Aecia<sup>1</sup> conicolous, subcorticular, distinct, sphaeroid, or rarely confluent and cerebroid (Pl. VI, fig. B).

Sori firm but not bladderly, 1–5 mm. in diameter, averaging 2.6 mm.;<sup>2</sup> peridia circumscissile, soon falling away in flakes, leaving rounded spore masses, and often broken lacerate edges at their margin; peridial cells usually in one to two layers, overlapping, irregularly sphaeroid to clavate or fusiform, basal cells longer and narrower than the apical ones, 14–32 by 28–100  $\mu$ , averaging 22 by 56  $\mu$  with walls 4–10  $\mu$  thick, averaging 6  $\mu$  inner walls finely striated with tubercular outgrowths; aeciospores sphaeroid to ovoid or ellipsoid, 12–25 by 19–51  $\mu$ , averaging 18 by 35  $\mu$ , (Patouillard gives a range of 12–20 by 25–40  $\mu$ ), with walls 2–5  $\mu$  thick, averaging 3  $\mu$ , coarsely verrucose with dense, somewhat deciduous tubercles, with a thickened smooth spot at the base.

II. Uredinia<sup>3</sup> hypophyllous, rarely epiphyllous, usually subepidermal, from a limited mycelium, in chlorotic (never reddened) areas of the leaves, single, scattered or clustered, sometimes confluent, irregularly sphaeroidal, rupturing irregularly at the apex; peridium evident but evanescent; urediniospores capucine yellow in mass, fading when old, sphaeroid to obovoid, rarely ellipsoid, 11–25 by 16–36  $\mu$ , averaging 18 by 25  $\mu$ , with walls 2–5  $\mu$  thick, averaging 3  $\mu$ , echinulate on the surface.

III. Telial columns hypophyllous, filiform, chestnut to bay, 2.8 to 6 mm. long, averaging 4.3 mm., 70–150  $\mu$  in diameter, averaging 112  $\mu$ ; teliospores oblong to fusiform, 10–23 by 20–50  $\mu$ , averaging 16 by 34  $\mu$  walls tinged with brown 2–6  $\mu$  thick, averaging 4  $\mu$ .

<sup>1</sup> The pycnia are described from type specimen F. P. No. 25402, collected by N. Rex Hunt, September 28, 1918, and the aecia from F. P. No. 36152 by Otto Schoenberg August 10, 1920, both on cones of *Pinus chihuahuana* near Portal, Arizona.

<sup>2</sup> The dimensions of sori in this description are based on not less than 50 measurements, and of spores, not less than 100.

<sup>3</sup> The type specimens for the uredinia and telia are F. P. No. 29666 on the leaves of *Quercus hypoleuca* collected by N. Rex Hunt, September 28, 1918, near Portal, Arizona. These and the type for the pycnia are deposited in the Office of Pathological Collections, United States Department of Agriculture, Washington, D. C.

## COLLECTIONS OF THE FUNGUS

*Cronartium conigenum* has been collected according to our records, in Arizona and Mexico as follows:

I. On *Pinus chihuahuana*; Mexico, by Maury in 1891; Arizona, Chiricahua Mountains, by A. H. Zachau, July 17, August 20, and September 2, 1909, also April 24, June 27 and August 27, 1914; Canille, by W. T. Doherty of the Forest Service July 12, 1914; near Portal, by N. Rex Hunt, May 26 and September 28 (Type *hypocynia*), 1918; also by A. J. Abbott, July 26, 1918, and by Otto Schenck, July 22 and August 10, 1920.

II. III. On *Quercus emoryi*; Arizona, near Portal, by A. J. Abbott, August 20, 1918; and by N. Rex Hunt, April 26, and September 28, 1918.

On *Quercus hypoleuca*; Arizona, near Portal, by N. Rex Hunt, September 28, 1918 (Type).

In addition to these specimens, collections have been made from the artificially infected trees of species of oak and chestnut at Washington, D. C., previously mentioned in this paper.

A COMPARISON OF THE MORPHOLOGY OF CRONARTIUM CONIGENUM  
AND C. STROBILINUM

<i>C. conigenum</i>	<i>C. strobilinum</i>
Infected cones living two or more growing seasons.	Infected cones dying the first growing season.
I. AECIOSORI usually distinct and separate.	AECIOSORI usually becoming confluent and continuous.
PERIDIUM erumpent, conspicuous.	PERIDIUM submerged, inconspicuous.
PERIDIAL CELLS 22 by 56 $\mu$ <sup>1</sup>	PERIDIAL CELLS 15-35 $\mu$
AECIOSPORES 18 by 35 $\mu$ <sup>2</sup>	AECIOSPORES 15 by 25 $\mu$
II UREDINIOSORI in chlorotic areas of leaf tissue	UREDINIOSORI in reddened areas of leaf tissue
UREDINIOSPORES 18 by 25 $\mu$ <sup>3</sup>	UREDINIOSPORES 15 by 20 $\mu$
III TELIAL COLUMNS chestnut to bay, 0.11 by 4.3 mm.	TELIAL COLUMNS mummy brown to black, 0.15 by 3.8 mm.
TELIOPORES 16 by 34 $\mu$	TELIOPORES 15 by 30 $\mu$

<sup>1</sup> In this table the average of not less than 100 measurements are given in each instance.

<sup>2</sup> Measurements for the aeciospores of *Cronartium cerebrum* (Peck) Hedgec. and Long, 15 by 26  $\mu$ , for *C. fusiforme* (Peck) Hedgec. and Hunt, 16 by 25  $\mu$ .

<sup>3</sup> Measurements for the urediniospores of *Cronartium cerebrum*, 12 by 20  $\mu$ , and for those of *C. fusiforme*, 13 by 18  $\mu$ .

OFFICE OF INVESTIGATIONS IN FOREST PATHOLOGY,  
BUREAU OF PLANT INDUSTRY,  
WASHINGTON, D. C.



## DESCRIPTION OF PLATE V

Fig. A. Size of a one year hypertrophied cone infected with *Cronartium strobilinum* as compared with, uninfected cones of the same age. Cones collected from *Pinus palustris*, Dunedin, Fla. 1919. One-half natural size.

Fig. B. Hypertrophied cone showing aeciospore masses of *C. strobilinum* after the cortex above the aecial area has fallen away. The white flakes or flaps at the apex of the cone belong not to the rust but to a saprophytic fungus growing on the scales of the diseased cone. Specimen F. P. No. 29105, on *P. heterophylla*, Dunedin, Fla., 1918. One-half natural size.

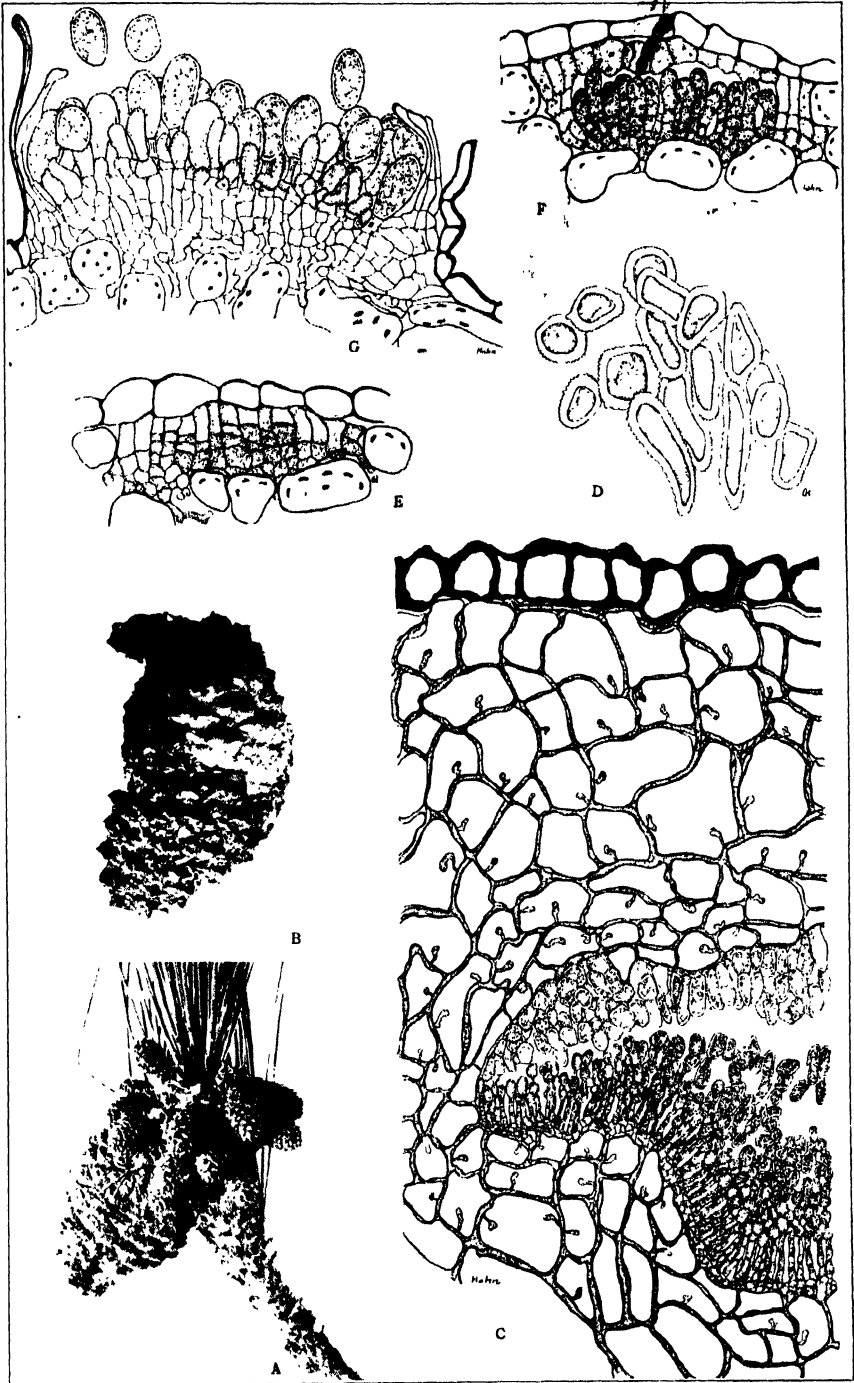
Fig. C. Cross section of infection of cone scale tissue of *P. palustris* showing the deep seated character of the aecial area of *C. strobilinum*. The peridium, which never becomes extruded, is shown as a definite multiple layer of cells above the aeciospores. Approximately  $\times 180$ . All drawings made with camera lucida by Glenn G. Hahn:

Fig. D. Peridial cells of *C. strobilinum*, approximately  $\times 400$ .

Fig. E. Early stages of peridium and urediniospore formation of *C. strobilinum*. Approximately  $\times 400$ .

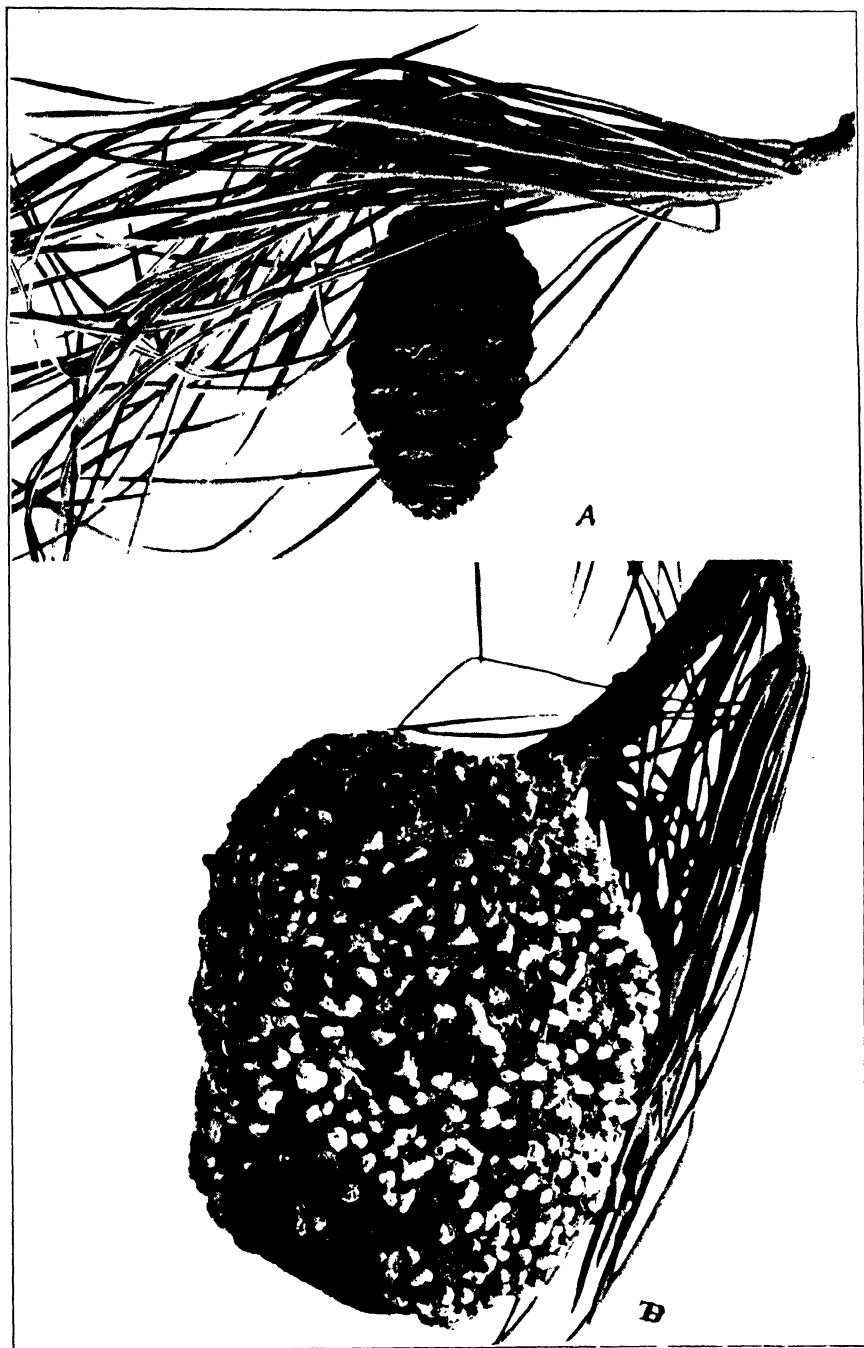
Fig. F. Separation of peridium from developing urediniospores of *C. strobilinum*. Approximately  $\times 400$ .

Fig. G. Mature uredinium of *C. strobilinum* showing urediniospores and ruptured and shriveled remnants of peridium  $\times 400$ .



*CRONARTIUM STROBINUM*





*CRONARTIUM CONIGENUM*

Fig. A. An immature two-year old cone of *Pinus chihuahuana*.

Fig. B. A diseased cone of *Pinus chihuahuana* bearing mature aecia.  
(Both figures about one-half natural size.)



## YELLOW DWARF OF POTATOES

MORTIER F. BARRUS AND CHARLES C. CHUPP

WITH PLATES VII AND VIII AND ONE FIGURE IN THE TEXT

A disease of potatoes probably new to science has made its appearance in New York State. It was first noticed by the senior writer in the summer of 1917 in Green Mountain stock in a field near Cadyville, Clinton County, New York. Only a few hills of diseased plants here and there in the field were observed at the time. They were quite different in appearance from those affected with any other disease he had observed before, the one or two stalks the plants possessed being dwarfed but stocky and the foliage having a marked yellowish tinge. Some of the plants were dying from the top down. On digging into a hill a few small irregular tubers were observed which, on being cut open, revealed a decided necrotic condition of the flesh in the form of rusty specks or areas most pronounced at the bud end of the tuber.

This disease was observed in the same locality the following year and during every year since. At the time of the conference of pathologists on Long Island in June, 1919, the disease was observed at Mattituck in a field of Green Mountains planted with seed obtained from Clinton County. Many of the pathologists present saw the disease there but none of them were acquainted with it. The following year the junior author observed the disease in Warren, Rensselaer, and Washington Counties and it was reported from other localities by members of our staff. At the present time it has been located with certainty in the following twenty counties: Cayuga, Clinton, Dutchess, Essex, Franklin, Fulton, Jefferson, Lewis, Madison, Oneida, Onondaga, Oswego, Otsego, Rensselaer, Saratoga, Steuben, Suffolk, Tompkins, Washington, and Warren (Fig. 1). It appears to be well distributed in the counties to the north and east. The disease has been observed on the following varieties: Green Mountain, Mills Pride, Norcross, Gold Coin, Goldson, Surprise, American Giant, Manistee, Dibles Russet, Rural Russet, No. 9, Sir Walter Raleigh, Heavyweight, Carmen, White Rose, Burpee's Early, Robson's Seedlings, and Mammoth Prolific. None of these seem to show any degree of resistance.

Plants were found showing all stages of the disease from those on which symptoms were scarcely distinguishable to those very much dwarfed. The most marked characters as seen in the field are the dwarfed condition and yellow color because of which the name "yellow dwarf" was given by Dr. F. M. Blodgett. There are other characters that enable one to distinguish it from other diseases with which it may be confused. The stalks of plants are shorter than those of healthy

plants but have about the same diameter except toward the top where it is greater, giving such plants a stockier appearance. The growing apex dies early and later the axillary buds of the upper part are also killed. The stalk becomes yellowish green in color throughout. A longitudinal section of the entire length of a stalk reveals the presence in the upper portion of rusty colored specks in the pith and cortex at the nodes. In some plants this specking may be found in the tissues of the internodes as well. The specks may be few in number but usually are very abundant in plants in which the disease has progressed to any extent. This discoloration of the cortex often becomes sufficiently pronounced so that it can be observed from the outside through the epidermis. Sections of the axillary and terminal shoots indicate the nature of their death as all tissues are rusty brown and necrotic. These

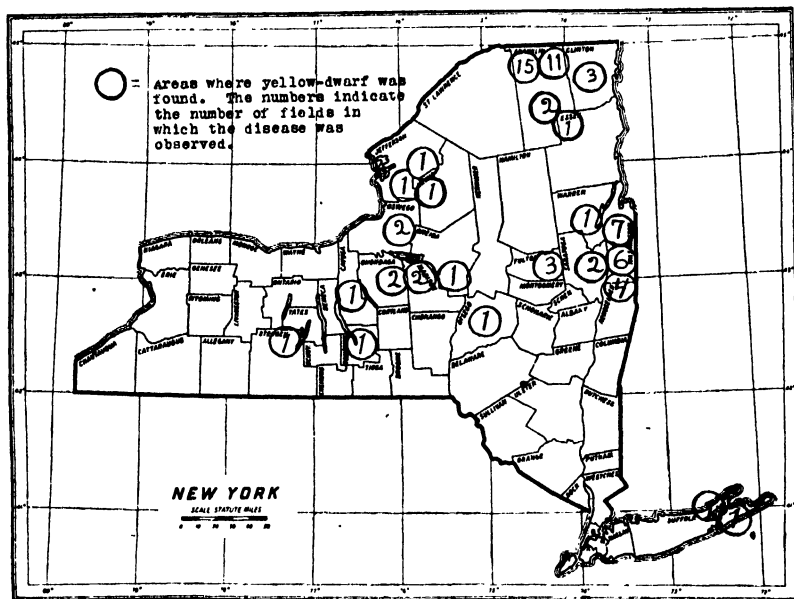


FIG. 1. DISTRIBUTION OF YELLOW-DWARF IN NEW YORK STATE. DUTCHESS COUNTY ALSO SHOULD BE INDICATED.

tissues at the lower part of the stalk rarely show any such discolored areas at any time in the progress of the disease.

The leaflets of affected plants, especially those toward the top, have a tendency to roll upward from the base to the tip and this symptom might confuse the disease with leaf roll were it not for the other symptoms. On blue sprout varieties, such rolled leaflets take on a purplish color where the under surfaces are exposed to direct light. The yellow color predominates as the disease becomes more pronounced until the entire top shows it to a greater or less extent. On some leaves the lower leaflets lose all green color and the yellow takes on a lighter tint

becoming almost white. Affected plants show a greater tendency to tip and marginal leaf burn than do normal ones.

During 1920 the junior author noted in Warren County a type of extreme dwarfing which was not so prevalent during 1921. This absence of dwarfing may have resulted from unfavorable conditions for potato growth during 1921 so that badly diseased plants died before the usual time for inspection. The mature plants were only two or three inches tall, the tops being tufted or rosette-like, while the stems were as thick as those of full grown healthy plants. The leaves were drawn or corrugated as if seriously affected with mosaic, and early began to show the yellow discoloration. This condition might have been mistaken, in some cases, for a type known as curly dwarf were it not for the symptoms present in the tubers.

One could not, however, confuse yellow dwarf with other diseases after examining the tubers. It is true that, in common with leaf roll, affected plants usually produce tubers, small and few in number (Pl. VII, fig. 1). Very commonly, too, these are sessile or are formed on short stolons (Pl. VIII, fig. 1). Occasionally the tubers are irregular, sometimes knobby and cracked, sometimes large and smooth tubers are produced (Pl. VII, figs. 2, 3). The cracked condition is very characteristic of the disease. The cracks first appear in the sides of the tuber and later may extend from one end to the other (Pl. VII, fig. 4). Often several cracks occur radiating from the bud end. They have the appearance of being due to a greater growth within the tuber than was taking place on the outside. That a stress actually exists within affected tubers before cracks appear is shown by the readiness with which such tubers will split open when a knife is stuck into them. Sometimes the cracking occurs near the middle and extends longitudinally up and down. The depth of the cracks varies but commonly they extend inward five mm. or more. The exposed area usually becomes covered with a corky layer before harvest so that loss of moisture is prevented as well as infection from rot producing organisms through these wounds. Occasionally cracks may be found in the interior of the tuber, especially tubers that have been in storage. This shows as a hollow area of greater or less extent.

Another character of the tubers affected with this disease that may be depended on in diagnosis is the discoloration of the flesh. This usually appears as rusty brown specks or areas surrounding the pith area but occurring more or less throughout the surrounding medullary regions or perimedullary tissue and in the cortex. These specks or areas are few and scattered in some tubers and numerous or massed in others but such discoloration does not appear as streaks. Longitudinal sections of the tuber show that the discolored areas are most pronounced



at the middle and often occur nearly to the bud end of the tuber, but they are usually absent at the stem end as seen from an examination of tubers in the field, differing in this particular from wilt diseases. Sections of tubers taken from storage show that the discolored areas occasionally occur nearly or quite to the stem end (Pl. VII, figs. 2, 3).

Not all tubers from affected plants show internal discoloration. Occasional tubers have been found in which there is no marked appearance of an abnormal condition either externally or internally. Sometimes tubers are found that show only one or a few small grayish-brown spots in the cortical region. One farmer, who said he had known the disease to exist in his fields for ten years at least, was able to sort out affected tubers from his bin without cutting them even when they appeared normal in size and shape. "They look different," he said, and a careful comparison of such tubers with healthy ones of the same variety indicated a greater prominence of the lenticels of affected tubers. This condition, however, is not sufficiently marked to attract attention unless a comparison is made.

When one or more shoots of a hill are healthy and others of the same hill are affected the tubers attached to the healthy shoots, occasionally at least, are also healthy while those attached to the affected shoots are usually diseased, so that healthy and affected tubers may be found in the same hill (Pl. VII, fig. 2).

The seed piece has nearly always been found intact in the case of yellow dwarf plants that have been observed in the field. This condition of the seed piece has also been reported as of common occurrence with leaf roll plants. Tubers distinctly affected with yellow dwarf when planted in the greenhouse and out of doors have usually decayed but this may be due to the more advanced stage of the disease in such tubers than in those observed under field conditions.

Professor F. C. Stewart has kindly furnished us his notes on a potato disease observed at various localities in Ontario County as early as 1914. There was a distinct specking of the parenchyma of the stems in the vicinity of the nodes and cases were observed in which the shoots appeared to have died from the tip backwards. There was, however, no yellowing of the foliage nor cracking of the tubers although a necrosis of the vascular tissue of the tuber at the stem end was a rather constant accompanying symptom.

Growers who have been endeavoring to eradicate the disease by roguing affected plants are able to detect the disease on the vines at an early stage. These early symptoms were pointed out to the authors and were found to consist of a slight yellowing of the foliage at the apex of a branch with some rosetting. Frequently in such cases the terminal bud was dead even at this stage and plants were found occasionally whose tubers, while possibly normal in size, had already

cracked and, as sections revealed, showed the characteristic internal discolored areas of the disease. Often only one branch showed these symptoms, the others appearing healthy. The cortex and pith of the affected branch showed, in most cases, the rusty specks in the upper nodes while the healthy appearing ones were free from them. The freedom of the lower portion of such affected branches from any appearance of disease would lead one to the conclusion that an infection had taken place above ground, from some outside source of inoculum, except for the presence of the symptoms in the tubers of such plants. Plants have been found in the latter part of the season showing these early symptoms which seems to indicate that no matter where infection occurs the tuber is not always the source of inoculum.

A large percentage of the tubers from affected plants are worthless for table stock or for seed. Plants affected late in the season produce some marketable tubers but even such of these as show internal discolorations usually are discarded. The percentage of affected plants in most fields that have been under observation is small however, ranging in most cases from a trace to 1 or 2 per cent. Some fields have shown 5 to 10 per cent and a few were observed where more than 20 per cent of the plants were affected. It can be seen that the disease may become serious. We do not know whether the larger area in which it has been found in 1920 represents a recent distribution or a failure to recognize it in preceding years. Efforts made by growers to eradicate it have been unsuccessful although they have kept it under control.

The writers have not carried out experiments to any extent to demonstrate the manner in which the causal agent, if any, is carried. They have been fortunate in being able to inspect a large number of variety tests conducted in various parts of the state in which a number of varieties of certified seed from different localities as well as a few local varieties have been planted in each test. Only one inspection of each test was made and the various tests were inspected at different times during the season. Table I shows the percentage of yellow dwarf found in each of the certified lots of each test. It seems reasonable to conclude from these data that the causal agent existed in the soil as the disease appeared in a majority of the lots in five tests located in counties where the disease has been found to be most prevalent while in the other localities where these same stocks were used the disease does not occur on any of them except in a few cases where a single variety was affected. The local varieties used in those tests where the certified stock showed the disease so generally had some affected plants in nearly every case. None were found among local varieties in the other tests except those in Steuben County. It should not be concluded from these data that the disease cannot be communicated with the seed. It

TABLE 1—Localities where yellow dwarf occurred and did not occur in eleven stocks planted in various localities in variety test 1921.

County	Locality	Percentage of Yellow dwarf in stock from										
		Gardner & Son Tully Rur. Rus.	M. Porter Rod- man Sir Walter Raleigh	M. Thomp- son Avoca Godson	C. C. Cornue Avoca No. 9	F. Gibbs Fillmore Hyt.	K. Liver- more, Honey- oye Falls Rur. Rus.	S. Robinson Marathon Gr. Mt.	A. Reeves Lysander Green Mts.	O. Rob- son Hall Robson Seedling	E. Merihew Marath- on Nor- cross	D. Nettle- ton Marath- on Gr. Mt.
Fulton	Broadalbin	0	0	0	0	-	-	-	-	0	0	0
	Broadalbin	1.25	0	1.25	2.5	0	2.5	3.75	2.5	2.5	0	-
	Northampton	-	-	1	-	0	1	1	0	0	0	-
	Vail Mills	0	0	0	0	0	0	0	0	0	0	0
Green	Mayfield	-	3.5	5	1	0	0	2	6.5	.5	-	-
	Hunter	0	0	-	0	0	0	-	-	0	-	0
	Adams	0	0	-	0	0	0	0	0	0	0	0
	Carthage	1	1	1	1	1	2	2	1	1	6	3
Jefferson	Rodman	0	0	0	0	0	0	0	0	0	0	0
	Copenhagen	0	2	0	0	0	0	0	0	0	0	0
	Martinsburg	0	0	0	0	0	0	0	0	0	-	-
	Stanley	0	0	-	0	0	0	-	-	0	-	-
Ontario	Victor	0	0	-	0	0	0	-	-	0	-	-
	Naples	0	0	-	0	0	0	-	-	0	-	-
	Clifton Sps.	0	0	-	0	0	0	-	-	0	-	-
	Ballston Lk.	0	0	-	0	0	0	0	0	0	0	0
Saratoga	Saratoga Sps.	0	0	-	0	0	0	0	0	0	0	0

TABLE I (Continued).

County	Locality	Percentage of yellow dwarf in stock form										
		Gardner & Son Tully Rus.	M. Porter Rodman Sir Walter Raleigh	M. Thompson Avoca Goldson	C. C. Cornue Avoca No. 9	F. Gibbs Fillmore Hywt.	K. Livermore, Honey-oye Falis Rus.	S. Robinson Marath-on Gr. Mt.	A. Reeves Lysander Green Mts.	O. Robson Hall Robson Seedling	E. Merihew Marath-on	D. Nettle-ton Marath-on Gr. Mt.
Seneca	Lodi	2	0	-	0	0	0	-	-	0	-	-
	Waterloo	0	0	-	0	0	0	-	-	0	-	-
Steuben	Prattsburg	0	-	0	0	0	-	-	-	-	-	0
	Naples	0	-	0	0	0	-	-	-	-	-	0
	Arkport	1.5	-	-	0	0	0	-	-	-	-	-
	Arkport	-	-	0	0	-	0	0	0	0	0	-
	Bath	0	-	0	0	0	-	-	0	-	-	0
	Wheeler	-	0	0	0	-	0	0	0	1	0	-
Tompkins	Savona	-	0	0	0	-	0	0	0	0	0	-
	Avoca	-	0	0	0	-	0	1	0	0	0	-
	Brookton	0	0	-	0	0	0	-	-	0	-	-
	Freeville	0	0	-	0	0	0	-	-	0	-	-
Warren	Lake George	-	-	0	-	-	-	0	.5	-	1	.5
	Chestertown	-	-	-	.5	-	-	0	0	-	C	.5
Wyoming	Bliss	-	0	-	0	0	0	-	-	0	-	-
	Gainesville	-	0	-	0	0	0	-	-	0	-	-

TABLE II.—Percentage of yellow dwarf in certain rows as shown in variety tests conducted during 1921 in Washington, Franklin, and Suffolk Counties. All other rows showed no yellow dwarf.

Location of test	Source of Seed		Row no.	Variety	Percent yellow dwarf
	Grower	Address			
W. W. Parish near Salem, N. Y. 23 sources of seed each except one duplicated, planted 100 hills to a row	Allan F. Hand	Greenwich, N. Y.	1	Dibbles Russet	1
	" "	" "	24	" "	1
	T. Cummings	" "	2	Rural Russet	1
	" "	" "	25	" "	1
	F. B. Nelson	Salem, N. Y.	5	Manistee	4
	" "	" "	28	" "	5
	W. W. Parish	W. Rupert, Vt.	6	Sir Walter Raleigh	12
	" "	" "	29	" "	4
	T. Cummings	Greenwich, N. Y.	8	Norcross	2
	" "	" "	31	" "	1
	W. S. Collins	North Creek, N. Y.	15	Surprise	1
Allan F. Hand Greenwich, N. Y. 20 sources of seed planted in rows 137 ft. long	F. S. Hollenbeck	Tully, N. Y.	2	Rural Russet	1
	W. S. Collins	North Creek, N. Y.	5	Surprise	1
	A. F. Hand	Greenwich, N. Y.	9	Dibbles Russet	4
	P. Billings	" "	11	White Sprout	3
	F. B. Nelson	Salem, N. Y.	16	Manistee	2
	W. W. Parish	W. Rupert, Vt.	17	Sir Walter Raleigh	12

TABLE II continued

Location of test	Source of Seed		Row no.	Variety	Percent yellow dwarf
	Growers	Growers			
D. E. Martin Gabriels, N. Y. 24 sources of seed 100 hills of each planted in a row	Frank Delosh "	Brushton, N. Y. "	7 19	Carmen "	1 1
	Lake Placid Co. " M. Thompson " Bruce Cottrell	Lake Placid Club, N. Y. " " " Avoca, N. Y. " Homer, N. Y.	27 36 38 75 39	Green Mountain " Goldson " Green Mountain	5 0 1 1 1
H. J. Reeves Mattituck, N. Y. 62 sources of seed 1 pk. of each planted in test	Lake Placid Co. " "	Lake Placid Club, N. Y. " " "	27 36	Green Mountain " "	3 5

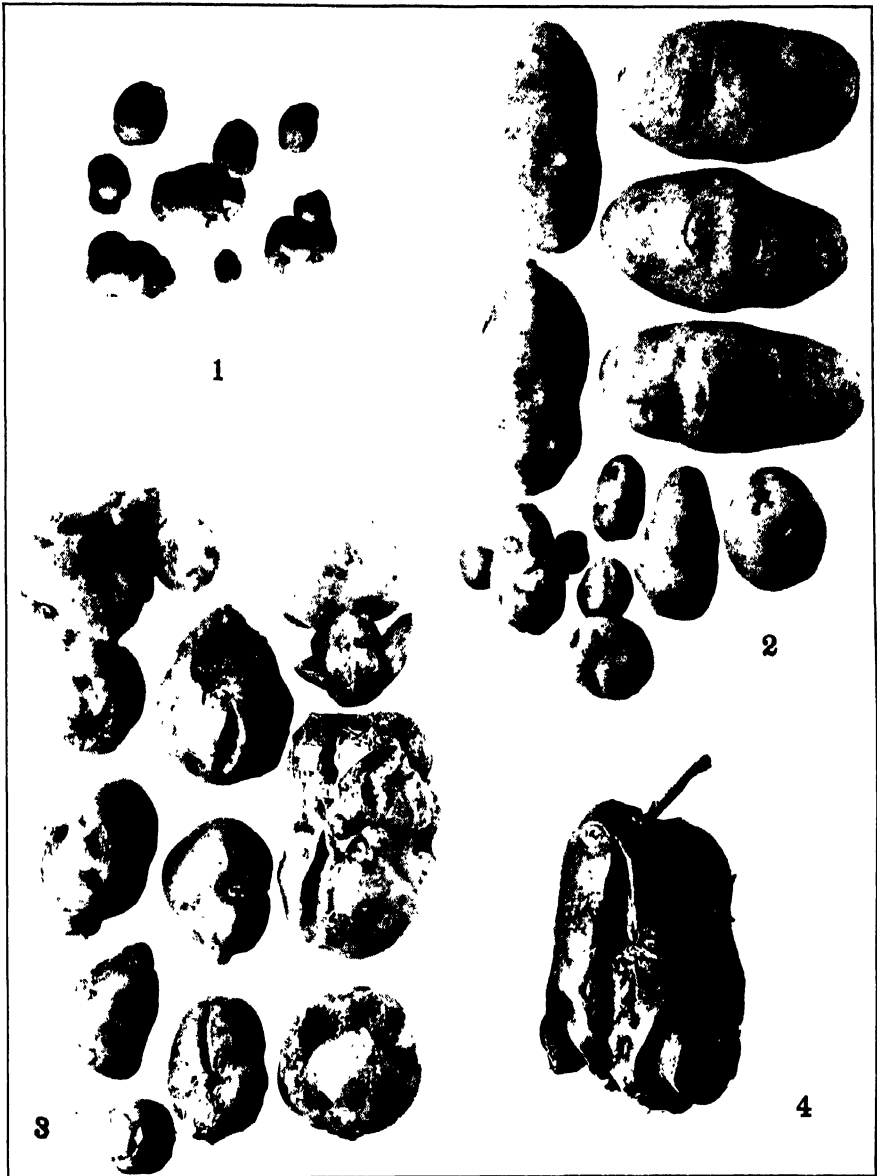
is probable that it did not exist to any extent in the certified stock used, for it was selected stock in most cases and nearly all affected tubers, if such were present, would have been graded out before being shipped or upon cutting them at planting. Of course, it is possible that the causal agent could be carried with contaminated soil adhering to the tubers. It is known that the disease occurred to a slight extent in 1921 in the fields of Mr. Thompson at Avoca, N. Y., and may have existed in his stock in 1920. There is no record of its existence in the fields of those growing the other certified stock used in these tests. Soil and weather conditions are factors that may have some importance in connection with the appearance of the disease but they have not been considered here.

Attempts were made a number of times to determine whether affected tubers will produce affected plants. The smaller affected tubers have a tendency to dry rot within a month or two after digging and before the rest period is completed (Pl. VIII, fig. 4). Those that remained comparatively sound when planted in most cases either produced no sprouts or only feeble ones, the seed piece drying to a spongy brown mass. The vines that succeeded in coming through the ground were dwarfed and spindling, not having the symptoms described above as typical for the disease. These did not live long enough to produce tubers.

A few of the variety tests, however, gave indications that the disease is communicated with the tuber. Table 2 gives data to support this statement. One cannot examine such tests where certain stocks showed the disease in replicated rows while others did not, without believing that it was carried with the seed rather than that infection took place from the soil in which the seed was planted.

The ways and means of infection should not be difficult to determine but are not given because no serious effort has been made in this direction. For this reason, too, the writers are unable to present suggestions concerning the cause of the disease and the many other features of interest and importance in relation to it. Further observations may determine that it is but a different manifestation of symptoms produced by an organism already known as pathogenic to potatoes. It is thought desirable to present these observations now rather than wait until a complete study has been made in order that others may become aware of the existence and of the symptoms of the disease and may be on the lookout for it.

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YELLOW DWARF OF POTATOES

FIG. 1. Yield from one badly diseased hill. No marketable tubers.

FIG. 2. Yield from a hill, a part of the vines being dwarfed. Five of the tubers are normal.

FIG. 3. Yield from one diseased hill. Each tuber shows cracking or other deformity.

FIG. 4. Severe, but characteristic cracking of a diseased tuber.







## YELLOW DWARF OF POTATOES

FIG. 1. Basal part of a potato stem affected with yellow-dwarf. Observe the thick stem, sessile tubers and persistent seed piece!

FIG. 2. Cross sections of tubers showing typical lesions of yellow-dwarf. Unlike the condition found in wilt, the discoloration is seldom found in the fibro-vascular bundles.

FIG. 3. Longitudinal sections of tubers similar to those in figure 2.

FIG. 4. Rot of tubers following yellow-dwarf.



# LEAF ROLL, MOSAIC AND CERTAIN OTHER RELATED DISEASES IN IDAHO

CHAS. W. HUNGERFORD

WITH PLATE IX

Potato leaf roll and mosaic have become of very great importance in various potato growing sections of the United States in the last few years. An examination of the Plant Disease Survey Reports of the United States Department of Agriculture reveals the fact that these diseases have not only caused more loss each year in the eastern states but also have been increasing to an alarming extent in the West. This reported increase may be due to a certain extent to the fact that they are becoming better known and more generally recognized, but there is very good evidence that these insidious diseases have spread very rapidly throughout the country.

Our records in Idaho show that both leaf roll and mosaic have gradually increased both in number of fields infected and in severity of infection during the three years since 1919, when records of the occurrence of these diseases in the state were first available. In 1919 the loss in Idaho from mosaic was estimated at one-half of one per cent., in 1920 at one per cent. and in 1921 at over three per cent. The same gradual increase seems to hold true for all of the potato producing states in the West.

When the writer first noted the mosaic disease in Idaho in 1919, it occurred on the variety known as the Netted Gem. There was some question as to whether the true mosaic was present. Mottling as it appears on the Bliss Triumph and on certain other varieties was not present. The characteristic crinkled and corrugated appearance, however, suggested mosaic. Schultz and others (8) have since listed the Netted Gem as among the varieties in which mosaic does not normally develop mottling. At the suggestion of Dr. I. E. Melhus, who visited the Idaho Experiment Station in 1919, progeny from several plants which had these symptoms were planted in the greenhouse. Under greenhouse conditions faint but unmistakable mottling appeared.

Numerous fields were also noted the same year in northern Idaho where from one to 20 per cent. of the plants showed unmistakable symptoms of leaf roll. As very little was known regarding the possible importance of these diseases in the northwest and the means by which they are disseminated under our conditions, some preliminary trials were made in 1920 with selected seed from various parts of the state.

## FIELD TEST WITH LEAF ROLL AND MOSAIC

Tubers from plants infected with mosaic and from other plants showing the typical leaf roll condition were planted at Moscow on the experimental farm. Tubers from plants which were apparently healthy were planted as checks. The following table gives the results of these preliminary tests. Those in the series from C1 to C12 were from apparently healthy plants in fields where very little mosaic or leaf roll was found. C14, C15 and C16 were from apparently healthy plants taken

TABLE 1

*Showing the source, variety, and yield of marketable tubers obtained from potatoes selected for seed from apparently disease-free and from mosaic and leaf roll plants*

No.	Source	Variety	Number Hills Planted	Weight of Tubers		Ave. Wt. of Marketable Tubers per 10 Hills
				Market-able	Culls	
C1	Moscow	Netted Gem	13	19	0	15
C2	"	" "	41	44	5	11½
C3	"	" "	40	42	6	10½
C4	"	" "	30	54	4	18
C5	"	" "	28	43	2	17
C6	"	" "	22	39	3	17
C7	"	" "	21	33	3	16
C8	Troy	" "	20	30	4	15
C9	"	" "	34	53	2	15
C10	"	" "	34	51	4	15
C11	Rupert	" "	19	15	2	8
C12	Caldwell	" "	24	39	6	16¼
C13	Rigby	" "	22	18	4	8
C14	Moscow	" "	12	0	1	0
C15	"	" "	20	0	5	0
C16	"	" "	13	0	4	0
M1	"	" "	29	0	1	0
M2	"	Gold Coin	15	0	5	0
M3	Rupert	Betted Gem	9	6	2	6
M4	King Hill	Ida. Rural	5	0	3	0
M5	Rigby	Netted Gem	58	23	14	4
LR1	Moscow	Gold Coin	21	8	3	3½
LR2	"	Netted Gem	20	4	3	2
LR3	Princeton	" "	17	6	4	3½

from a field where from 75 to 80 per cent. of the plants were in the advanced stages of either mosaic or leaf roll. Those from M1 to M5 were selected from plants with definite mosaic symptoms. Those from LR1 to LR3 from plants in the early stages of leaf roll. Each number represents the tubers from one plant only, except in numbers M1, M2, M5, LR1, LR2 and LR3, where tubers from several hills having like symptoms were planted together. The source indicates the place in

the state where seed was secured. Caldwell, Rupert, Rigby and King Hill are in the irrigated section of southern Idaho. Moscow, Troy and Princeton are in the non-irrigated section of North Idaho.

It will be noted that in M1 although 29 hills were planted only one pound of cull tubers was harvested. These plants were very badly diseased and although the symptoms in the plants from which they were originally selected were clearly mosaic, the progeny showed symptoms of mosaic, leaf roll and curly dwarf. The one pound of culls from this lot was planted again in 1921, and practically no tubers were formed on any of the plants. The fact that C14, C15 and C16, which were apparently quite healthy plants selected from a badly diseased field, yielded no marketable tubers in 1920, and that all of the progeny in these three lots were badly diseased with either mosaic, leaf roll or both, would indicate that these diseases may cause very serious decrease in yield even the first year after infection has taken place.

#### RUSSET DWARF

Another potato disease which has been under observation in Idaho for the last three years and which appears to be somewhat similar to mosaic has been called russet dwarf. This disease was first called to the author's attention by Professor E. R. Bennett, Extension Horticulturist of the University of Idaho, in 1919. During that year the disease was noted in a large number of fields of Idaho Rural potatoes in Canyon County. In some fields as high as 35 per cent. of the plants were infected. This disease has previously been named and a brief description published (1, 2).

#### SYMPTOMS OF RUSSET DWARF

In the field, diseased plants have somewhat the appearance seen in certain types of mosaic. They are of a somewhat lighter green color than normal and only about two-thirds as large as healthy plants growing under the same conditions. The most conspicuous symptoms of infected plants as seen from some distance is the general rusty appearance of the leaves, especially the lower ones. In the more advanced stages of the disease many of the lower leaves fall off. A careful examination of the lower leaves of an infected plant reveals the fact that the veins of the lower side have a water soaked appearance in the earlier stages and finally turn brown. Death soon follows to a portion or all of the leaf and leaves fall off progressively from the ground up until in some

cases plants have been noted where the lower half of the plant was completely defoliated. Dark brown streaks are also present on the petioles and stems of the lower part of the plant. Later in the season the rest of the plant becomes affected and death of the entire plant often results. A careful check on the yield of healthy and diseased plants in the same field revealed the fact that the yield was reduced from one-half to two-thirds on the average. Plate IX, D shows a hill of Idaho Rurals affected with russet dwarf in comparison with a healthy hill.

When progeny from these diseased plants are grown in the greenhouse the symptoms noted above are much more striking and others appear which were not noted in the field. Diseased plants can be picked out as soon as they emerge from the ground. The first leaves are quite uniformly crimped or buckled downward at the tip. (Pl. IX, A). This characteristic is shown in all of the earlier leaves, some of them being so deformed as to be broader than long. Instead of the dwarfing which was noted in the field, the stalks often become elongated until they are sometimes several inches taller than healthy plants of the same age. (Pl. IX, E). The later leaves which develop are more normal in shape but are still somewhat crinkled. When the plants are four to five inches high, streaks with a water-soaked appearance begin to appear on the veins of the underside of the affected leaves. These streaks often start at the outer margin of the leaves extending in on the larger veins. They soon turn brown and a considerable portion of the veining on the under side of the leaf may become involved. Streaks of a similar nature may appear anywhere on the main stem or on the petioles of the leaves. Often a spot appears about half way up on the under side of the midrib where the leaf appears to be constricted. Frequently the midrib may crack at this point. Under greenhouse conditions the lower leaves begin to drop off rather early. The petiole of the affected leaf may become constricted near the stem and the leaf may fall before it has lost much of its green color. (Pl. IX, A). In some cases one-half of the leaf may be effected while the other half appears normal. This dropping of the leaves may take place progressively up the stem until only a rosette of leaves remains at the top. (Pl. IX, E). New growth sometimes starts at the axil of the leaf which has fallen. This in turn soon dies and drops off. (Pl. IX, B).

#### COMPARISON WITH OTHER DISEASES RECENTLY DESCRIBED

Murphy (4, 5) has recently reported a disease which he called leaf-drop which according to his description and photographs is very similar to russet dwarf. There are several points in which the symptoms of these two diseases do not agree, however. Murphy states that affected

plants are poorly covered with foliage and consist in the majority of cases of a single shoot. This is not the case with russet dwarf, in which there are the normal number of leaves and stems but on account of the reduced size of the leaves the plants often appear to have much less foliage. He also states that there is a slight browning of the vascular ring in the tubers from diseased plants. This characteristic has not been constantly associated with tubers from plants infected with russet dwarf. One symptom which is constant for russet dwarf, and which Murphy states is the one which separates leaf-drop from all other types, is the death and dropping of the leaves one after the other from the ground up. Murphy also states that the yield from plants diseased with leaf-drop is extremely low, being from one ounce to nothing at all per plant. This is not true of russet dwarf which we have noted under our conditions. In most cases the yield as stated above is reduced from one-half to two-thirds.

Russet dwarf also appears to have some characteristic in common with the disease which Murphy (4) has called crinkle, especially in the puckering and downward curling of the younger leaves as described above. In other ways it does not correspond at all with his description of this disease. Quanjer (6) also describes crinkle and speaks of this same characteristic. He also states that a bronze tinge prevails in some varieties affected with this disease. This description might apply to the characteristic russetting which appears in russet dwarf late in the season. Orton (7) in discussing the streak disease of potatoes makes the following comment: "There is for example, a disease not uncommon in our trial grounds, marked by weak, erect stems, from which frequently the spotted leaves fall prematurely from the ground upward, so that a palm-tree like effect is produced." This description might well apply to russet dwarf as we have seen it in the greenhouse.

Krantz and Bisby (3) in discussing symptoms of mosaic state: "The lower part of the plant is often entirely defoliated in severe cases of mosaic dwarf in Minnesota." From the author's own observations in the Northwest and in the light of recent work, in Canada by Murphy and in Holland by Quanjer, it would seem that more than one disease has been described under the name mosaic in the United States.

#### TRANSMISSION OF RUSSET DWARF

It has been definitely shown that this disease is carried from year to year by means of tubers from diseased plants. This has been true in all of a large number of trials. Preliminary trials also indicate that the disease may be transmitted from plant to plant in the field. Idaho Rural potatoes were secured from a badly diseased field at Parma, Idaho, in 1919. Three lots of seed were secured. First, plants were



selected which were in the advanced stages of russet dwarf. Second, plants were selected which were adjacent to russet dwarf plants but which were themselves apparently free from the disease. In the third place, apparently healthy plants were selected which were at least five feet from any plants which were affected with russet dwarf. The first lot produced plants which were all affected with russet dwarf in 1920, the second produced plants which were over half of them affected, and the third lot produced plants over one-third of which were affected with the disease.

A grower in the northern part of Idaho secured seed from the southern part of the state from a field where there was a considerable amount of russet dwarf in 1919. In 1920 about 35 per cent. of the plants grown from this seed developed the disease. The grower rogued the field very carefully throughout the season and used the same seed another year. In 1921 about 15 per cent. of the plants developed the disease. It seems rather evident then that this disease as well as mosaic is infectious and that diseased plants may infect healthy ones in the field. Experiments are under way at the present time to learn if possible the agents which transmit the disease under Idaho conditions.

Numerous attempts have been made to isolate a causal organism from diseased plants. So far it has not been possible to prove the pathogenicity of any organism so isolated. A study of the pathological anatomy of diseased plants is also being undertaken.

#### CALICO

A condition which is locally known as "calico" is fairly common in irrigated sections of Idaho, Utah and Washington. Mention of this disease has already been made in two previous publications (1, 2). This disease is characterized by a pronounced variegation of the leaves of the plants. In extreme cases as much as half of the surface of the leaf may be almost entirely lacking in chlorophyll. The plants appear normal in every other way. Calico is much more pronounced early in the season many of the leaves appearing to develop chlorophyll in these chlorotic areas at about blossoming time. All evidence to date seems to show that this condition is inheritable but not infectious. Progeny from calicoed plants have in all cases produced plants with some of the leaves having this pronounced variegation. When tubers from plants showing this variegation were planted in the greenhouse, the symptoms which developed were similar to those noted in the field except that in some cases the chlorotic areas turned brown when the plants were about half grown. Later the whole leaf might become affected and the death of the leaf sometimes followed. Plate—, C shows a typical calico plant.



LEAF ROLL AND MOSAIC OF POTATOES

Fig. A. Idaho Rural potato plant showing early stage of russet dwarf in the greenhouse. Note curling at the tips of the leaves.

Fig. B. Idaho Rural potato plant affected with russet dwarf in the greenhouse. Note progressive dropping of the leaves from the ground upward and the new growth starting in axil of the lower leaves which have fallen.

Fig. C. Idaho Rural potato plant affected with calico. Note variegation on lower leaves in the foreground at X.

Fig. D. Healthy hill (at left) and russet dwarf (at right) under field conditions.

Fig. E. Healthy hill (at right) and russet dwarf (at left) under greenhouse conditions.



The yield of tubers does not appear to be materially reduced by this disease and the amount of infection in any field observed has never been over 3 per cent.

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## THE DISSEMINATION OF PEACH YELLOWS AND LITTLE PEACH.<sup>1</sup>

MEL T. COOK

These two diseases of the peach have been the subject of very earnest study for many years and although a great deal of important data has been accumulated we do not yet know either the causes of these two diseases or the methods by which they are disseminated. Furthermore, the symptoms are not well understood. It is very generally recognized by plant pathologists that very definite information on these three points is of the very greatest importance in developing satisfactory methods for the control of any disease.

For several years the writer has been cooperating with Prof. M. A. Blake and Mr. C. H. Connors of the Department of Horticulture of the New Jersey Agricultural Experiment Station in the study of these two diseases. The complete and detailed results of these studies have been published in bulletin number 356, January 1922. In these studies it was demonstrated that the budding of the nursery stock was one of the very important factors in the spread of these diseases. The nurserymen and the growers who bud their own stock practice two methods of securing their bud wood: 1 by selecting an apparently healthy bearing tree and 2 by budding from row to row, i. e. the taking of bud wood from vigorous young stock. A careful study shows that these diseases may be very readily distributed by either of the above methods. In the first case the nurseryman may make his selection for the purpose of securing stock of a certain variety or because he wishes to perpetuate a character which has appeared in some individual tree. The second method is followed because of its convenience and because it saves both time and labor, or because the nurseryman is satisfied with his stock and hesitates to make a new start.

In making studies of this kind it must be remembered that symptoms which are the same or similar to the recognized yellows and little peach symptoms may result from other causes: Some of the most common causes are 1, partial or complete girdling by borers, mice or other small animals; 2, injuries due to climatic factors, especially winter injuries at or just below the surface of the ground; 3, mechanical injuries, especially label wires which are sometimes left on the tree; 4, improper soil fertilization; 5, lack of proper cultivation; 6, other plant diseases especially those which cause partial or complete girdling; 7, unfavorable soil conditions; 8, defoliation due to improper use of sprays.

<sup>1</sup> Paper No. 63, of the Journal series, New Jersey Agricultural Experiment Stations, Department of Plant Pathology.

The studies of the author and his associates brought out the following points which are discussed more fully in the bulletin.

1. These diseases can be transmitted by using buds from diseased trees; the greater the severity of the disease in the scion tree, the more rapid its development in the young tree. The diseases can be transmitted by buds taken just before the development of the symptoms in the scion tree. The diseases can be transmitted by buds taken from apparently healthy branches of a diseased tree. The disease can be transmitted although the buds fail to grow. The disease can be transmitted by inserting a piece of bark from a diseased tree into a healthy one. In the case of June budding from a diseased tree, the disease does not develop until the second year. In one of our experiments a tree budded with yellows developed little peach.

2. Juices transferred from diseased to healthy trees did not carry the disease.

3. There is some proof that the disease is not carried by the pollen.

4. Pits from diseased trees very rarely germinate. It is very doubtful if the few that do germinate carry the disease.

5. The diseased are most likely to appear in orchards that have come into full bearing.

6. The diseases are likely to appear as epidemics.

7. The diseases are sometimes more severe on certain blocks of stock than on others.

8. The removal of a branch showing either of these diseases from a tree which is otherwise apparently healthy does not lead to the recovery of the tree although the tree may not show symptoms again until the second year following the removal of the branch.

9. These diseases are not equally prevalent every year. We had a very severe outbreak of yellows at the Vineland, N. J., experimental orchard in 1907 and a very severe outbreak of both diseases in 1919-20; in the latter outbreak the little peach was much more abundant than the yellows.

10. Blocks of trees of the same variety but from different nurseries do not develop these diseases to the same degree although planted side by side. Blocks of trees of the same variety and from the same nursery may develop different degrees of these diseases on different soil areas although separated by not more than one quarter of a mile.

11. The time and rate of incubation of these diseases dating from time of budding is not known. Trees budded from trees in which either of these diseases is very severe usually develop the disease the following year, while those budded from trees in which the disease is mild may not show symptoms for three, four or more years. Trees budded from a branch showing symptoms of either of these diseases will develop the

disease much more quickly than trees budded from the apparently healthy part of the same tree.

It will be readily seen from the above statements that although we do not know all the possibilities of transmission of these diseases, we do know that they can be readily transmitted by budding. We also know that the diseases may incubate as much as four years before the first symptoms appear. We also know that the early symptoms are very likely to be over-looked by those who have not made a special study of these diseases. It will be readily seen that it is possible for the most careful budder to select buds from diseased trees in which the symptoms are as yet not evident or only slightly developed. It will also be seen that young trees in the nursery may be diseased but not show the symptoms and that the row to row method of budding may result in carrying the disease from year to year and from old to new stock without the disease appearing in the nursery.

The fact that one or the other of these diseases sometimes develops in certain blocks of stock far in advance of other blocks in the same orchard leads the writer to suspect that nursery stock is sometimes a source of dissemination. Furthermore, growers sometimes take bud wood from trees which ripen their fruit a little early, thinking they have found an early variety and not realizing that the early maturity is a symptom of yellows. In cases of this kind, which have come to the writer's attention, the trees have developed the disease just as they were coming into bearing or very soon after.

The practice of the nurseryman cannot be criticised and the method of budding from row to row is commendable but these methods do not necessarily guarantee healthy stock. The trees from which the bud wood is taken should be kept under observation for a long period of time before the cutting of the bud wood so as to determine their characters and freedom from disease. They should be kept under observation and if any tree develops either disease within that time, the stock from that tree should be destroyed.

A few years ago the New Jersey Agricultural Experiment Station offered the nurserymen of the State bud wood from trees that had been kept under close observation from time of planting in the orchards. Several nurserymen accepted this offer. Of course, it has been impossible to follow these trees and we cannot give the results. Furthermore, the conditions arising from the world war made it necessary for us to discontinue the practice. Would a method of this kind followed out for a number of years, tend to reduce these diseases?

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# COMPARATIVE SUSCEPTIBILITY OF EUROPEAN AND AMERICAN VARIETIES OF CUCUMBERS TO BACTERIAL WILT

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In the course of an investigation of possible varietal resistance of cucumbers to cucurbit mosaic during the summer of 1921, a number of European and American varieties of pickling cucumbers were grown together at Madison, Wisconsin. The mosaic disease attacked all of these varieties with equal severity, but those from Europe proved extremely susceptible to bacterial wilt, *B. tracheiphilus* Erw. Sm., when compared to varieties grown in this country.

The plat used for this work was approximately one-half acre in size and had been used for cucurbit mosaic disease studies for the past five years, the disease being especially severe there each season. Bacterial wilt, however, had never caused serious losses prior to 1921. Seeds of commercial varieties of pickling cucumbers were obtained from Holland, France, and Germany, and planted in alternate rows with seeds of standard American varieties. As only a limited amount of the European seed was available, in some cases two of these varieties were planted in a single row, the foreign varieties being located in the center of the plat and several additional rows of American varieties at each end.

The planting was done on June 7, except rows 1 to 3, which were planted on June 20. The seeds were planted in drills in rows six feet apart, and the plants later thinned to a distance of fifteen inches. After the final thinning a record was made of the number of plants in each row, but, owing to the irregular shape of the plat and the uneven stand of plants in certain rows, no effort was made to secure a uniform number of plants per row. Bacterial wilt first developed about July 7, but no exact record was made of the wilted plants until July 18. After this date the plat was inspected at intervals of four to six days and records made of all plants which showed symptoms of bacterial wilt infection. The inspections were discontinued after August 18 as the combined attacks of mosaic and wilt made further detailed records of little value. The striped beetle, *Diabrotica vittata*, was present in considerable numbers throughout the season, being unusually numerous from June 15 to July 30. The insects were uniformly distributed over the plat and attacked all varieties with equal avidity. The following table shows the extreme variation in the amount of wilt on the European and American varieties.



TABLE 1. *Comparative susceptibility of European and American varieties of cucumbers to bacterial wilt.*

(European varieties with data in italics)

ROW *	VARIETY	NUMBER OF PLANTS	PERCENTAGE OF WILT AT DIFFERENT DATES				
			JULY 18	JULY 25	JULY 30	AUG. 6	AUG. 18
1	Heinz Pickling	110	0.0	0.0	0.5	3.0	22.0
2	Heinz Pickling	104	0.5	1.0	1.0	4.0	23.0
3	Heinz Pickling	111	0.0	1.0	5.0	13.5	23.0
6	Heinz Pickling	122	1.0	7.0	10.0	11.5	24.5
7	Heinz Pickling	124	1.0	5.0	8.0	12.0	23.0
8	Heinz Pickling	104	1.0	8.5	12.0	16.0	27.0
9	<i>Short Green Parisian<sup>1</sup></i>	<i>50</i>	<i>4.0</i>	<i>26.0</i>	<i>40.0</i>	<i>80.0</i>	<i>90.0</i>
	<i>Short Green Early<sup>1</sup></i>	<i>59</i>	<i>6.5</i>	<i>20.0</i>	<i>59.0</i>	<i>74.5</i>	<i>85.0</i>
10	Snow's Pickling	136	1.5	5.0	11.0	12.5	22.0
11	<i>Oblong Green Pickling<sup>2</sup></i>	<i>62</i>	<i>7.0</i>	<i>47.5</i>	<i>59.5</i>	<i>77.5</i>	<i>94.0</i>
12	Snow's Pickling	113	1.5	6.0	9.0	11.5	37.5
13	<i>Half Long Prolific<sup>2</sup></i>	<i>65</i>	<i>5.5</i>	<i>17.0</i>	<i>46.0</i>	<i>80.0</i>	<i>92.5</i>
	<i>King William<sup>2</sup></i>	<i>59</i>	<i>6.0</i>	<i>16.5</i>	<i>39.0</i>	<i>84.0</i>	<i>100.0</i>
14	Fordhook Pickling	128	3.0	5.5	21.0	25.5	47.5
15	<i>Vert petit de Paris<sup>3</sup></i>	<i>74</i>	<i>12.0</i>	<i>21.5</i>	<i>53.0</i>	<i>70.0</i>	<i>92.0</i>
	<i>Cornichon de Meaux<sup>3</sup></i>	<i>54</i>	<i>6.5</i>	<i>22.0</i>	<i>43.5</i>	<i>76.0</i>	<i>93.0</i>
16	Fordhook Pickling	118	1.0	6.5	17.5	22.5	27.0
17	<i>Vert petit de Paris<sup>3</sup></i>	<i>33</i>	<i>12.0</i>	<i>36.5</i>	<i>78.0</i>	<i>87.0</i>	<i>100.0</i>
	<i>Cornichon de Toulouse<sup>3</sup></i>	<i>32</i>	<i>6.5</i>	<i>25.0</i>	<i>37.5</i>	<i>65.5</i>	<i>85.0</i>
19	Chicago Pickling	113	2.0	13.0	15.0	22.0	31.0
20	Fordhook Pickling	99	1.5	18.0	19.0	21.0	37.0
21	Jersey Pickling	99	0.5	9.0	9.0	15.0	30.0
22	Chicago Pickling	113	0.0	10.5	10.5	12.5	25.5
24	Chicago Pickling	96	1.0	18.5	19.0	23.0	33.5
25	Snow's Pickling	104	0.5	14.5	24.0	28.5	35.5
26	Heinz Pickling	90	1.0	12.0	12.5	13.5	33.5
27	Heinz Pickling	91	2.5	12.0	12.5	17.5	37.0
28	Heinz Pickling	66	3.0	12.0	12.5	16.5	41.0
Averages for American varieties			1.2	8.7	12.0	15.8	30.5
Averages for European varieties			7.6	25.8	50.6	77.1	92.4

\* Rows 4, 5, 18, and 23, not planted to cucumbers.

<sup>1</sup> German<sup>2</sup> Dutch<sup>3</sup> French

It will be seen that all of the varieties from Europe showed a much greater percentage of wilt infection from the time the disease first appeared. On July 30, the average infection on the European varieties was approximately four times that on those of American origin. On August 18, but a few plants remained alive in the rows planted with

European seed, while the American varieties, although suffering considerable losses from the disease, would still have produced profitable crops were it not for the injury from mosaic. The high percentage of infection was strikingly uniform on all of the European varieties and consistently low in the case of those of American origin.

The data on wilt infection in this instance is complicated by the fact that mosaic was also spreading through the plot throughout the season. Although all of the plants were eventually affected, the spread of the mosaic disease was unusually slow and the infection was evenly distributed over all of the rows. Up to July 30, not over 8 per cent of the plants were mosaic diseased, and by this time the variation in susceptibility to wilt had been amply demonstrated.

The outbreak of bacterial wilt on this plot, which was somewhat isolated from other cucumber fields, was far more severe than any observed in other fields at Madison or at other points in Wisconsin and Illinois during the season. Bacterial wilt, however, was more severe in these states during 1921 than for several years previous, although serious losses occurred only in rare instances.

Additional evidence of the susceptibility of certain of the European varieties was secured in two other instances. The German varieties Short Green Early and Short Green Parisian, together with the varieties Oblong Pickling and King William from Holland, were planted in single rows adjoining a large block of the American variety, Heinz Pickling. The wilt was much less severe in this field, but approximately 40 per cent of the foreign plants were infected on August 20, as compared with an infection of less than 20 per cent on the American variety. On this date not over 1 per cent of the plants were mosaic diseased. A field of about one acre at Marengo, Illinois, planted with the foreign variety King William, showed an estimated infection of 15 per cent on August 9, while five fields in the vicinity planted with American varieties showed an average of less than 6 per cent.

It was noted that all of the European varieties were extremely susceptible to the hot, dry weather which prevailed during much of the summer, the older leaves developing a noticeable yellowing and curling toward the edges. This type of injury was found in nearly all fields in Illinois and Wisconsin, but was much more marked on the European varieties. These plants also wilted much more rapidly after infection than did those of the American varieties, the latter often showing evidence of local infections where the organism had seemingly failed to progress further. In the case of the European varieties, however, the infection nearly always seemed to involve the entire plant with considerable rapidity. It has seemed possible that plants produced from seed grown under the milder climatic conditions of western Europe might

be more susceptible to a disease of the wilt type when grown under the more intense heat and dry weather which prevailed in the Central States during the last season.

Although bacterial wilt is reported as occurring in Europe, the greater resistance of the American varieties may also be due partly to a greater prevalence of the disease in this country and a consequent development of comparatively resistant sorts through a gradual elimination of the more susceptible strains.

## A NEW ALTERNARIA SPOT OF TOMATOES IN CALIFORNIA<sup>1</sup>

BRUCE DOUGLAS

WITH ONE FIGURE IN THE TEXT

In the fall of 1913 the writer first noticed a spotting of tomato fruit which was apparently distinct from other forms of tomato diseases that had been previously reported in California. This spotting has been noted in several localities in southern California but its study has been confined to the Whittier district, one five acre patch of tomatoes being studied carefully through two seasons.

The trouble appears soon after the first fall rains or heavy fogs and generally increases from then until the end of the season. On the fruit it is characterized by brown circular spots which become depressed as they increase in size. Later the surface of the spot is covered with a black velvety mass of *Alternaria* spores. The spots remain quite firm until secondary fungi and bacteria of a saprophytic nature enter, when the fruit goes down with a watery decay.

### ISOLATION OF THE FUNGUS

In the fall of 1913 a species of *Alternaria* was isolated from the spots and by inoculation with spores similar spots were produced on sound fruit. In the fall of 1914 the fungus was again isolated fifteen different times from naturally occurring spots. Cultures obtained from plating out by the single spore method were used for the later inoculation experiments. For the isolation work Hiss media was used.

<sup>1</sup> The manuscript from which this brief account is extracted has been submitted with drawings and photographs to J. Rosenbaum and D. J. Mubraith, both of whom believe that the fungus as judged from the illustrations is neither *Macrosporium solani* nor *M. tomato*. Since the author of the paper is now a practising physician in Detroit and will not have opportunity to do further work on the disease, it is thought advisable to put this brief account of the work on record, with the suggestion that the disease may be different from any of those previously described on tomato.—H. S. Fawcett, Riverside, California.

In making cultures the following means were employed. (1) The skin of the fruits were flamed and pieces at the edge of diseased spots cut out and placed on the medium. (2) Other cultures were made from the fleshy part of the fruit under spots from which the epidermis had been cut away. The fungus was found at different depths varying from just under the epidermis to a little more than a quarter of an inch into the fleshy part of the fruit. (3) Still other cultures were made by drawing a sterile needle over the velvety mass of spores on the surface of a more mature spot. The fungus was readily obtained both from fruit fresh from the field and from that which had been placed in storage to ripen. The characters of spores of this fungus are shown in figure 1.



FIG. 1. SPORES OF AN ALTERNARIA FOUND ON TOMATOES IN CALIFORNIA

#### INOCULATION EXPERIMENTS

Inoculation experiments both on the fruit and on the foliage of the tomato were carried out. The method in which spores in large drops of water were placed on well sized green fruits just previous to the time they began to change color produced the most characteristic spots. Sterile water was added frequently during the first week so that the spores were not allowed to become dry. Inoculations of this character both in the open field and in moist chambers brought on spots in ten days to three weeks depending somewhat on the time it took the fruit to color and on the moisture conditions. Controls in which the same procedure was used with the exception that the spores of *Alternaria* were omitted, were allowed to remain for even longer than three week periods but in no case did spots appear where drops of

water without the spores had been placed. The *Alternaria* fungus was recovered in pure culture from a large number of artificially infected fruits but it was not found in the controls.

#### SPOTTING OF DETACHED LEAVES

Leaves from twenty-three varieties of tomatoes grown in the greenhouse were placed in moist chambers and sprayed with suspension of spores of this *Alternaria* fungus in sterile water. What appeared to be severe spotting from the *Alternaria* occurred with some varieties while others showed no effect. The fungus was reisolated from some of the spots. The varieties may be divided roughly into four groups according to the effects indicated at the end of three weeks.

Group I. Spotting severe: Beef Steak Los Angeles, Spark's Earliana, Stone, Trophy.

Group II. Spotting medium in amount: Acme, Beef Steak Whittier, Clark's Early Jewel, Crimson Cushion, Dwarf Champion, Dwarf Stone, June Pink, Livingstone's Favorite, Matchless, Perfection, Ponderosa, Yellow Peach, Yellow Pear, Yellow Plum.

Group III. Spotting slight: Beauty Livingstone, New Stone.

Group IV. No spotting: Atlantic Prize, Germain, Winter Queen, Golden Queen.

### EXPERIMENTS IN THE INFECTION OF *PINUS STROBUS* WITH *CRONARTIUM RIBICOLA*

(A PRELIMINARY STATEMENT)

HARLAN H. YORK AND WALTER H. SNELL

#### METHOD

About 500 potted seedlings of *Pinus strobus* of the seasons 1918 and 1921 were inoculated at North Conway, New Hampshire, in 1921. All of the inoculations were made in iceless refrigerators which were modifications of the one described by Hunt,<sup>1</sup> and which were placed near a small brook in a densely shaded swale in a pine woods. Freshly picked leaves of cultivated *Ribes nigrum* which bore an abundance of recently developed telia were used as a source of the inoculum.

Two methods of applying the inoculum were used, as follows: (1) The *Ribes* leaves as soon as gathered were supported 1 to 2 cm. above the needles of the seedlings on small sticks, before the telia had begun to germinate. (2) The telia were first allowed to germinate in moist chambers. The freshly gathered *Ribes* leaves were cut into pieces 1

<sup>1</sup>Hunt, N. Rex. The "iceless refrigerator" as an inoculation chamber. *Phytopath.* 9: 211-212. *Pl.* 12. 1919.

to 2 cm. x 1 cm. The pieces were then placed with the lower side upwards on a piece of ordinary window screen which was supported over a piece of absorbent cotton, saturated with water, in a covered Petri dish. When an abundance of sporidia had developed, the pieces of leaves were then lodged in the axils of the needles, of the seedlings. When the inoculations were made the seedling pines were placed in the refrigerators, sprayed with water and the inoculum applied. Then the plants were again sprayed with water and the refrigerators set in operation. During the entire time the seedlings were in the refrigerator droplets of water were present on the needles. After the seedlings were removed from the refrigerators, they were placed for a few days where they were shaded all day and then later moved to a garden where the pots were buried in the soil.

The temperature and humidity in the refrigerators were recorded by a properly checked Fries hygrothermograph. The latter was placed about 20 inches above the seedlings. The ground where they stood was thoroughly saturated with water. It is possible that the humidity in the immediate vicinity of the leaves was slightly higher and the temperature a little lower than where the hygrothermograph was located.

#### RESULTS

While it is not yet possible to give a full account of our inoculations enough evidence is already at hand to show that a large number of infections have occurred. The inoculated seedlings were examined November 21, 1921, by Dr. Perley Spaulding of the Office of Forest Pathology. He found typical yellow spots on the needles of 15 lots of the seedlings. A fascicle of needles from a 1918 seedling, No. 5299, and one entire 1921 seedling, No. 5317, were sent to Dr. R. H. Colley of the Office of Forest Pathology for examination. Colley has reported that the needles in both samples were thoroughly infected. In his report he states: "If anything, there are proportionately more individual infections in the needles of the 1921 seedling than in full grown leaves. In the former the spots were scattered generally along the whole length of the leaf." Eighty-five spots were counted on one needle of the 1918 seedling. The spots averaged about one per mm. in the infected areas. Substomatal vesicles were found in the leaves of the 1921 seedling. Both plants were from a series of 50 seedlings which were inoculated, beginning at 3:30 p. m. August 16, 1921. The inoculum was applied according to the method mentioned above. At the end of 38 hours, the Ribes leaves were destroyed and the seedling pines were removed from the refrigerators. During this time the average temperature and relative humidity in the refrigerators were respectively 65° F. and 94 per cent.

In a series of inoculations which were made on August 25, beginning at 2 a. m. the inoculum was applied according to the second method. At 2:30 p. m., 12½ hours later, on the same date, the inoculum was destroyed and the pine seedlings were removed from the refrigerator into the open air when the temperature and humidity were respectively 73° F. and 63 per cent for over one hour. The average temperature and relative humidity in the refrigerators during this experiment was 64° F. and 95 per cent.

At the beginning of this experiment, sporidia were removed from some of the pieces of leaves which were used for the inoculum and placed on needles of *Pinus strobus* which were then transferred to an empty iceless refrigerator where the temperature and humidity were 60° F. and 99 per cent. After one hour a few sporidia had begun to germinate.

No microscopic examination was made of the needles of the plants used in this series, yet yellow spots, which are typical of incipient infections, were present in great abundance. Such spots have not become visible in the controls. The latter consisted of 10 plants which were inoculated by scraping off telial columns with their sporidia and placing them on needles of plants which were left out in the open. The needles of the controls were slightly moist with dew. The plants were placed where they became fully exposed to bright sunlight about 8 a. m.

#### DISCUSSION AND SUMMARY

According to our observations which have been made in nature and on cultures of telia in damp chambers and in water, sporidia become fully developed from 5 to 6 hours after dry teliospores are brought under conditions favorable for their germination and the formation of sporidia. Considering this fact, together with the results of the second series of inoculations mentioned above, it might seem that at least 18½ hours of constant duration of such conditions of moisture and temperature as prevailed in our experiments are necessary for infection to occur on *Pinus strobus* in close proximity to *Ribes* on which sporidia are being produced.

Infection may occur within 12½ hours after the viable sporidia of *Cronartium ribicola* reach the needles of seedling *Pinus strobus* under such conditions of temperature and moisture as prevailed in the above mentioned experiments.

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# CELERY MOSAIC<sup>1</sup>

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WITH PLATE X AND ONE FIGURE IN THE TEXT

In August 1921, our attention was called to some stunted and malformed Golden Self Blanching celery plants which were collected near Newark, New Jersey, by Mr. Nissley, Extension Vegetable Specialist who reported this condition to be severe on the farm from which the diseased celery was collected. Some time in September, Prof. Schermerhorn reported a similar case in his experimental plots at the College Station, where several varieties were growing together in the same field. The experimental field of celery was examined at this time and the characteristic symptoms of a typical mosaic disease were evident. The mosaic condition seemed especially severe on the Newark Market, a so-called easy bleaching variety.

Throughout the fall season there was very little rainfall, and the celery crop was no doubt suffering from the drouth. It was at first suspected that the mosaic symptoms of the plants might be caused by the unfavorable moisture conditions that existed at this time. It was assumed, however, that if the latter suspicion was correct, the celery plant which developes and produces new leaf shoots from a central heart, would in time overcome and outgrow the mosaic symptoms, if reset on a moist and fertile soil.

Given favorable temperature, moisture and cultural advantages, the malformed condition continued to appear on new shoots of diseased plants, the old leaves retaining their mosaic symptoms. Furthermore, the new leaves grown out from reset diseased plants in the greenhouse since October have been diseased more severely up to January 15 than were the larger branches which developed the symptoms in the field, demonstrating that the condition was not induced by lack of moisture, but was a true mosaic.

## SYMPTOMS

The mosaic celery plants are very conspicuous, and readily distinguished from healthy ones. The entire plant is usually affected (Pl. X, fig. A). The foliage of the mosaic celery is sometimes drooping, wilting, or spreading. In most cases diseased foliage is nearly erect or straight becoming filiform and producing a bushy top (Pl. X, fig. B). As a general rule the leaves are shriveled and show numerous blister like pustules, which apparently do not burst, but affect the interior cell

<sup>1</sup> Paper No. 55 of the Journal Series. New Jersey Agricultural Experiment Station Department of Plant Pathology.



structure of the leaf to the extent of causing the malformation symptoms (Fig. 1). The diseased parts are very brittle, especially the young central branches. Force sufficient to pull up normal plants, will break off the tops of diseased plants, leaving the roots in the ground. There is no increased or decreased coloration observed in infected plants, either in the field or in the greenhouse.



FIG. 1 COMPARISON OF MOSAIC LEAVES WHICH SHOW BLISTERLIKE PUSTULES, WITH A HEALTHY LEAF.

#### INOCULATIONS

In studying celery diseases for the last three years, no such mosaic plants or any conditions similar to this disease have been observed on celery in this state, although aphids (*Myzus persicae*) have been common and sometimes severe. It has been necessary to constantly fumigate in greenhouses, where celery was grown for other investigational work but even after severe aphid infestation in other years no such disease was observed.

Diseased plants set in the greenhouse in October have continued to grow and a large number of new leaves have been produced. The disease was noted on all the new shoots from the heart in their earliest stages of development. In all cases where diseased plants were protected from aphids the mosaic symptoms continued to develop on the young leaves as fast as they were produced. This may indicate that

the causal substances is also present in the heart and root portions of the diseased plants.

By infesting healthy plants with aphids from diseased plants, the symptoms of mosaic were demonstrated in two weeks on Golden Self Blanching celery. In less than a month following infestation, the blister like pustules were very prominent. After a sufficient number of plants had been inoculated and the mosaic symptoms had developed the aphids were killed by fumigation in order to determine if the disease symptoms would appear in the young leaves which developed after the aphids were killed. It was demonstrated that the mosaic symptoms not only persisted, but the new leaves which developed from October to January, were more severely diseased and the symptoms more characteristic on Golden Self Blanching celery than on the so-called green varieties.

There were a large number of single diseased plants in the field growing in contact with healthy ones. Observations conducted over a period of 4 weeks, during which there was no aphid infection, gave no indications that the disease was spread by contact. It is known that aphids were present on the celery at one time during the growing season. No definite data is available to show how many times the plants were attacked nor how prevalent the aphids were on any occasion.

#### VARIETAL INFECTION

The disease was found this year (1921) in a varietal experimental plot. This is of particular interest, since the sources of seeds are traced to three different seed companies, and as indicated in table 1 nearly all the popular commercial varieties of this section were found to be

TABLE 1—*Showing the percentage of diseased celery plants in commercial seed of different varieties*

No.	Variety	No. plants examined	No. plants diseased	% diseased plants
1.	Newark Market.....	100	9	9.0
2.	" ".....	100	24	24.0
3.	" ".....	200	27	13.5
4.	" ".....	70	8	11.4
5.	Giant Pascal.....	150	1 (?)	0.66
6.	Golden Self Blanching.....	291	34	11.6
7.	O. A. C. Yellow.....	81	10	12.1
8.	Winter Queen.....	86	0	0.0
9.	Columbia.....	90	4	4.4
10.	Noll's Magnificent... ..	95	1	1.0

susceptible. It will be noted that in the first 100 plants examined in the Newark Market series, there were 9 diseased plants; while in the second 100 plants examined, there were 24 plants diseased. In the same plot, Giant Pascal, Golden Self Blanching, Oregon Agricultural College Yellow, Columbia, and Nolls Magnificent were also found to be diseased. Winter Queen was the only variety in which no disease was found. As is noted in the table, there is considerable variation in the infection of the different varieties. This may be due to lack of the spread of the infectious substance and not to resistance as the percentages of disease given in the table would indicate.

#### PROBABLE SOURCES OF INFECTION

For two years celery varieties have been tested on the same field plot. In 1920 the celery was examined in order to estimate the percentage of *Cercospora* and *Septoria* leaf spots in the different varieties. During that season, mosaic symptoms were not observed, but they occurred on the celery growing on the same soil in 1921. The seeds were germinated and seedling plants were grown in a greenhouse known to harbor the tomato mosaic. While there is yet no data to show that tomato mosaic is transmittable to celery, it might be added that such a relation seems probable since filiform and mosaic tomatoes grew adjacent to the celery experiment in 1921. Investigations are in progress to determine if the disease can be transmitted by other means, to celery and other plants. The relation of the disease to seed production and transmission by seeds is being studied. Since several months will be required to satisfactorily complete these details, it is thought advisable to present these data at the present time with the hope of throwing more light on the subject in a later paper.

The writer wishes to acknowledge suggestions from Dr. Mel T. Cook in connection with these studies.

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CELERY MOSAIC

FIG. A. A Mosaic Celery Plant.

FIG. B. Filiform and Mosaic leaves.



## PHYTOPATHOLOGICAL NOTES

*The relation of rain to the formaldehyde treatment of onion smut.* The object of this note is to emphasize a precaution that should be observed in applying formaldehyde for the control of onion smut.

During the spring of 1920, two onion-set growers in north western Indiana, co-operating with the Extension Department at Purdue University, used the formaldehyde drip method for the control of onion smut. The apparatus used was the type recommended by Walker (U. S. Dept. Agric., Farmers' bulletin 1060), consisting of a three gallon tank having a three-eighth's inch pipe to conduct the formaldehyde solution to the seed in the furrow. The solution used was made up at the rate of one pint of formaldehyde in sixteen gallons of water.

The treatment was successful in one case but much less so in the other. In the first field where white onions were grown 271.6 bushels per acre was the yield from the treated plots and 177.6 bushels from the untreated plots, an increase of 94 bushels per acre. In the second field the increase in yield due to the treatment was 60 bushels per acre in white onions and 20 bushels per acre in red onions.

The soil in each case is the same, a black sandy loam. Both growers have been raising onion sets for about the same length of time and there is no evidence that there is any variation in the degree of soil infestation by the smut in the locality. The most outstanding variant in these two cases was that of the weather conditions at the time of planting and subsequently.

In the first case where the results of the treatment were most satisfactory, the planting was done during the week of May 4. The grower was delayed in his sowing till this time by the continued rains of April. There was no rain during the first ten days of May. On the eleventh, twelfth and thirteenth there was a little more than an inch and on the seventeenth, eighteenth and nineteenth, nine-tenths of an inch more fell. It was six days after the onions were planted before it rained.

In the second case the grower planted his onions on Monday, April 26th. During the two weeks previous to the day of sowing 3.3 inches of rain fell in nine days. On the day that the seed was sown and the formaldehyde applied 0.28 inches fell as a light drizzle throughout the day. It also rained on April 27, 28 and 30. The total for these four days was 1.17 inches. In April there were 5.67 inches of rain in this region as compared with 2.01 inches in May.

One explanation of why the treatment was not successful in the latter case seems to be the frequent rains. It may be that the formaldehyde was rapidly leached away from the furrow containing the seed,

or diluted and not given an opportunity to disinfect. It may be possible also that the increased water content of the soil may have prevented the most efficient permeation of the soil by the gas and so greatly restricted the area of disinfection. Whatever the explanation, it would seem that the application of formaldehyde will not be as effective if made during rainy periods.—C. T. GREGORY.

*Crop Protection Institute fellowships.* In order to promote original research relative to the fungicidal and insecticidal properties of sulphur and the effects of sunlight, temperature and moisture on its action, the Crop Protection Institute expects to offer two fellowships yielding an income of \$2500.00 each. Training in chemistry and plant physiology is a prerequisite, and candidates should have demonstrated ability to undertake research efforts of a high type. Applications, accompanied by reprints of scientific articles and letters of recommendations, should be made immediately to the Crop Protection Institute, National Research Council, Washington, D. C. A statement explaining the purposes and scope of the projects and selection of research laboratory may be obtained on application.—W. C. O'KANE.

*Personals.*—Dr. P. J. Anderson of the Department of Botany, Massachusetts Agricultural College, has been transferred from the teaching staff of the college to be Research Professor of Botany in the Experiment Station. Dr. W. H. Davis of the University of Wisconsin has been appointed to the position made vacant on the teaching staff.

H. Atherton Lee, Mycologist, of the Department of Agriculture and Natural Resources, Government of the Philippine Islands, Manila, has accepted the position of Director of the Sugar Cane Investigations of the Philippine Sugar Centrals. Mr. Lee maintains his connections with the Bureau of Science as Mycologist.

Mr. Arthur C. Foster, formerly Extension Plant Pathologist for the North Carolina Agricultural College, was appointed Assistant Pathologist in the Bureau of Plant Industry January first. He is stationed at Sanford, Florida, where he is working in association with Dr. I. C. Jagger on diseases of lettuce, celery, and other Florida truck crops grown for the northern markets.

Mr. W. B. Tisdale of the University of Wisconsin recently accepted a position with the Florida Agricultural Experiment Station, Gainesville, where he is engaged in investigations of tobacco diseases.

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## BLACK ROT OF CARROTS CAUSED BY *ALTERNARIA* *RADICINA* N. SP.<sup>1</sup>

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WITH PLATE XI AND TWO FIGURES IN THE TEXT

During the winter of 1918-19 dealers at the New York market frequently complained of heavy losses due to decay of carrots in transit and storage, particularly in the case of stock that had been held in storage on Long Island farms. For this reason, early in April a trip was made by one of the writers to farms in the vicinity of Peconic and Orient, New York, for the purpose of studying local storage conditions.

The farmers living in this section of Long Island usually store their carrots in one of three ways; either in narrow pits, in mounds above ground or in cellars. During the course of the investigation it was found that irrespective of the method of storage used, a few of the carrots in most lots examined were either partially or entirely destroyed by a characteristic black rot. Examination of stock stored on farms in Danvers and South Peabody, Massachusetts, brought to light the fact that this disease is prevalent in these communities also.

The trouble, as it occurs on the root, is characterized by a progressive softening and blackening of the tissues. Infection seems to occur frequently at the crown, as a result of which the black decay extends down the core of the carrot, as shown by the photograph in plate XI, A. Many cases have also been observed where the decay had originated at other points on the surface of the carrot. Observations made on the crop in storage in the several localities mentioned in this paper indicate that although the disease is by no means as serious as that caused by *Sclerotinia* sp., it is, nevertheless, responsible for considerable loss each winter.

<sup>1</sup> The work discussed in this paper was done as a cooperative enterprise between the Office of Cotton, Truck, and Forage Crop Disease Investigations and the Bureau of Markets, United States Department of Agriculture, partly at Washington, D. C., and, through the courtesy of the Director, Dr. G. S. Gager, partly in the laboratories of the Brooklyn Botanic Garden, Brooklyn, N. Y.



On the above mentioned field trips, specimens were collected at Peconic, Southold, and Orient, New York, and at Danvers and Peabody, Massachusetts. The fungus was isolated from these specimens by transferring small pieces of infected tissue from a freshly exposed surface to hard potato agar tubes by means of sterilized instruments. Pure cultures of an undescribed fungus, which was later identified as a new species of *Alternaria*, were obtained from each carrot affected by the black rot. The fungus developed rapidly on potato agar, forming a dense, black mycelial growth over the surface, and after a few days produced conidiophores and conidia in great numbers.

#### INOCULATIONS OF CARROTS

In order to determine the relation of this organism to the decayed tissue from which it was isolated, a series of inoculations were made. Three dozen sound carrots were washed thoroughly with water and then subjected to surface sterilization. This consisted of washing the root quickly with 95 per cent alcohol, this treatment followed immediately by application of a mercuric chloride solution (1 to 1000). After the latter solution had been allowed to act for 5 minutes, the carrots were rinsed thoroughly in sterilized water. Three incisions were then made on each carrot by means of a flamed scalpel, and small masses of mycelium and spores taken from pure cultures of the fungus were inserted in the incisions on 28 specimens. The remaining 8 were not inoculated but were retained to serve as controls during the experiment. All of the carrots, both those which had been inoculated and the controls, were then placed in paraffin paper bags and held at room temperature for 3 weeks.

The decay developed rather slowly at the temperature of the laboratory during April. After 48 hours only a very small soft black spot had developed around the incisions on the inoculated carrots. No sign of decay had appeared around those on the control carrots. In fact, these 24 incisions closed up rapidly, drying out slightly, and the carrots remained uninfected during the experiment.

Twenty-one days after the inoculations were made, the carrots were removed from the paper bags and observations and photographs were made. Re-isolations from the decayed spots, carried out in the same way as the original isolations from the naturally infected roots, gave pure cultures of the *Alternaria* that was used for inoculation purposes. Every inoculation of the 84 resulted in infection, the characteristic black rot being produced in all cases. At the end of 3 weeks the decayed spots had developed to about an inch or more in diameter on the surface and half an inch to an inch in depth into the tissues of the root. Each spot was more or less depressed and its surface entirely covered by a

dense black mat of aerial mycelium with conidiophores bearing typical spores, singly or in groups in the manner of fungi generally referred to the genus *Macrosporium*. The tissues beneath the spot were softened and jet black in color. Plate XI, B, shows a carrot with 3 decayed spots resulting from inoculations. This should be compared with Plate XI, C, a photograph of one of the control carrots, which shows very clearly 2 uninfected incisions. Plate XI, D, is a photograph of median longitudinal section of the inoculated carrot shown in figure B. This last photograph shows the extent to which the fungus developed in the tissues of the root during a period of 21 days at room temperature.

#### INOCULATIONS OF THE FOLIAGE

During the summer of 1919, field inoculation experiments were carried out, to determine whether this fungus was able to cause a disease of the leaves as well as of the roots. It was also expected that such experiments would enable the writers to determine whether the black rot organism is related to *Macrosporium carotae* Ellis and Langlois, which causes a blight of the foliage.

Inoculations on the Danvers Half-long variety were made during cloudy weather in the last week in June. Spores from pure cultures of the black rot organism on potato agar were mixed with sterilized water and this suspension of spores was then brushed onto the foliage of the plants in two rows of carrots. Eight other rows of carrots in the experimental plot served as controls.

Within two weeks after inoculation, a disease had made its appearance on the inoculated plants. Small discolored areas appeared both on the leaves and the petioles. These spots were at first always brown in color, although in some cases they subsequently turned black. On the petiole the area of dead brown cells extended through the vascular tissues and thus cut off the water supply to the leaf. A wilt of the leaves resulted from these infections on the petioles. They turned yellow, then brown, and the conidia of the parasite developed in large numbers over the entire surface.

During the first month after the disease appeared on the inoculated rows, the control plants in the adjacent rows remained healthy and uninfected. But during early fall the fungus spread to these plants, with the result that the entire plot showed foliar lesions or entirely blighted leaves. The outer leaves of the plants blighted and fell to the ground, forming a mat of dead foliage between the rows.

Isolations were made from leaf lesions and from blighted leaves taken from these infected plants. Two methods were used: (1) spores were washed off in sterilized water and the suspensions thus obtained were plated out in Petri dishes; and (2) pieces of diseased tissues were sterilized by dipping in alcohol followed by mercuric chloride (1-1000), and then

placed on potato agar in Petri dishes. In either case the fungus obtained was similar in every way to that used in making the inoculations. Pure culture transfers were then made from these plates and the fungus was inoculated into carrots, the same precautions being observed against infections from the outside as outlined above for the previous experiments. The typical black rot developed around each inoculation.

Carrots which showed the blighted foliage were dug up, the tops removed to within an inch of the crown, and the entire surface of the root sterilized with alcohol and mercuric chloride. These roots were then placed in sterilized glass jars and held at room temperature. The same fungus again developed from the bases of the infected petioles and extended down into the center of the root, causing a black rot of the tissues.

Since the infection experiments on the leaves showed that the same organism which causes the black rot of the roots may also cause a disease of the foliage, a careful investigation was made of plants growing in the various fields where the carrots which showed this black rot last winter had been grown during the summer of 1918. Plants showing blighted leaves were collected on the farms at Peconic and Orient, New York, as well as Danvers and South Peabody, Massachusetts, and examined, in the laboratory for the presence of conidia similar to those developed on the inoculated plants. In every case conidia of a *Macrosporium* were found on the diseased leaves but these conidia did not resemble those developed on the inoculated plants but were readily recognizable as belonging to *Macrosporium carotae* Ellis and Langlois, being characterized by the presence of long, apical sub-hyaline appendages.

Many attempts were made during the summer and fall of 1919 to isolate *Macrosporium carotae* and to obtain pure cultures which might be used for inoculation purposes. While this fungus grew on potato agar and produced typical conidia, the development in culture was very sparse as compared with that of the black rot organism. In fact, the season passed before cultures suitable for inoculation work could be obtained.

#### THE FUNGUS

The conidial fructifications of the fungus developing on the surface of inoculated carrots, or in pure culture on agar media relatively rich in organic nutrients, as for example, potato dextrose agar, are of the type usually referred to the genus *Macrosporium*. On the substrata mentioned the production of aerial mycelium is relatively rapid and abundant. From the fuliginous or brownish hyphae that largely compose the dense black mats of aerial growth, the sporophores arise as short branches often several times septate and distinguished by their dark brown color. They bear at the tip usually one or two and more rarely three spores; the latter are attached directly to the sporophore and show no tendency toward a catenulate habit.

It may be mentioned that when the fungus is cultivated on substrata encouraging a rapid and abundant development, growth comes to a standstill comparatively early, stopping not unusually within 10 to 15 days. Such cessation of growth is, of course, familiar enough to students of nearly every group of fungi, and finds some plausible explanation in the accumulation of toxins. Whatever the explanation may be, the fact is quite obvious that fructifications developing under the conditions described, although often very abundant, are frequently not especially well developed. Thus more satisfactory results can often be obtained by the use of media containing little organic nutrient material on which growth is relatively scant, but which permit the moderate number of sporophores to continue development during a much longer period.

The black rot fungus was accordingly grown on tap water agar and Beijerinck's agar, neither containing any organic nutrients except such as were accidentally introduced. Drying out of the agar was prevented by incubating the Petri dishes in damp chambers. Microscopic examination of the cultures showed that at the end of 60 days the fungus was still growing. The fructifications, while in other details similar to those found on the richer substrata, showed not infrequently a catenulate arrangement of the spores, usually regarded as the distinctive characteristic of the genus *Alternaria* (Fig. 1, B, C, D.). Proliferation of secondary spores usually occurred at the tip of the primary spores, which were modified to form short subhyaline beaks. Such modification was not observed in spores that had not given rise to secondary spores. Occasionally the secondary structure was produced from one of the segments other than the distal one, in which case a short hyaline lateral process quite similar to the terminal modification supported the secondary spore.

The spores developed on carrots are straight, clavate, ellipsoid, obovoid, or turbinate; light brown to dark brown and dark olivaceous when fully mature; from 3 to 7 times transversely septate, with a longitudinal septum dividing some or all of the transverse segments not occupying a terminal position; measuring usually from  $17-20 \times 34-51 \mu$ . When developed on potato dextrose agar the spores generally measure considerably less. On tap water agar or Beijerinck's agar the spore measurements approximate those on the carrot, although showing in general a somewhat greater degree of variability. Spores like those shown in figure 1, A 1-3, probably represent nearly the maximum dimensions attained by the species,—width  $22\mu$  (Fig. 1, A 1-3),—length  $64 \mu$  (Fig. 1, A 2)—as well as nearly the greatest degree of septation, showing eight cross walls and two longitudinal septa in some of the transverse segments.

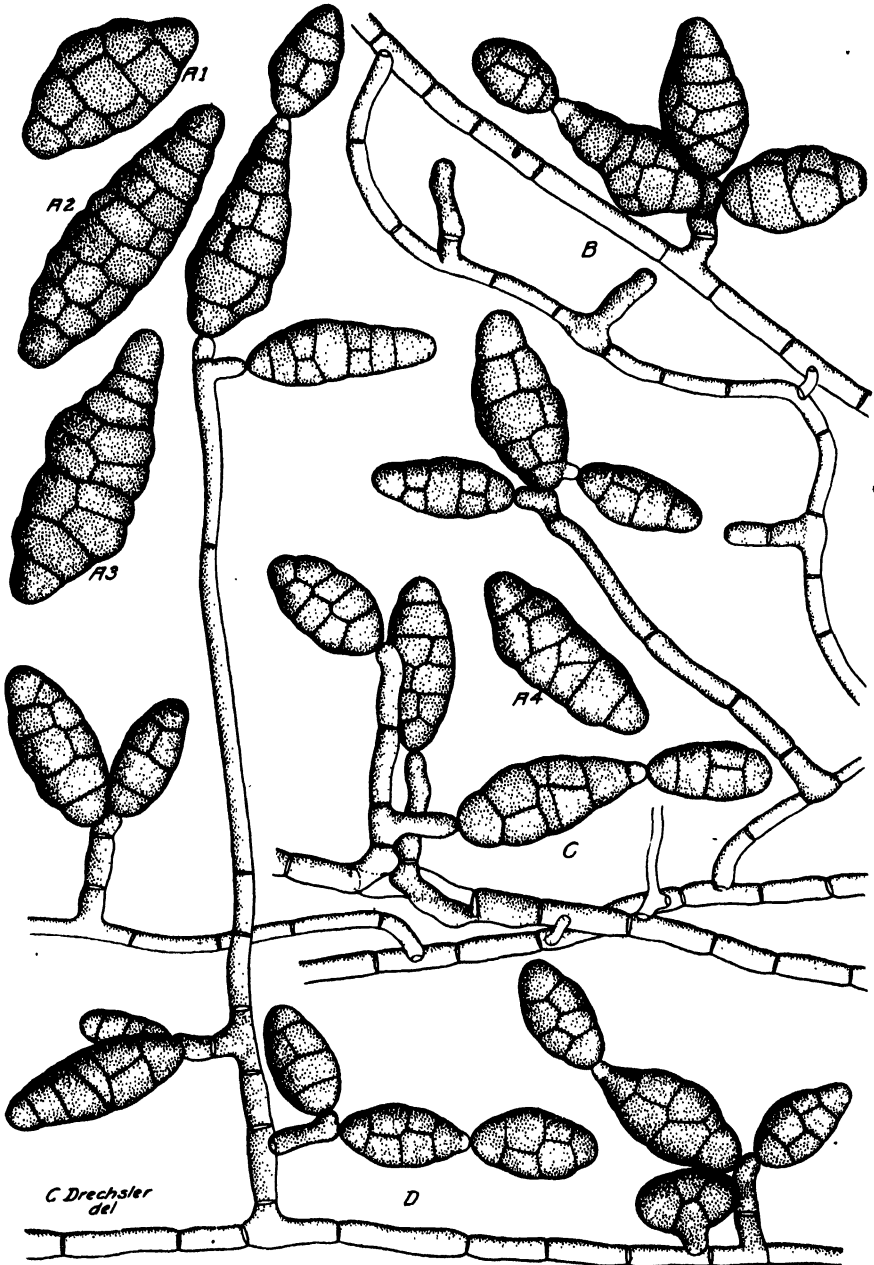


FIG. 1. *Alternaria radicina* from a culture on tap water agar incubated at a temperature of 15 to 20° C. x 535. A, 1-3. Spores illustrating approximate maximum dimensions and approximate maximum degree of septation. A, 4. Spore of average dimensions. B, C, D. Portions of mycelium showing darker colored short sporophores arising from more hyaline hyphae, the catenulate arrangement of some spores, and the tendency of the hyphae to anastomose with each other.

No report could be found in the literature of a root rot of carrots caused by a species of *Alternaria*, *Macrosporium*, or any of the allied genera. But Ellis and Langlois<sup>1</sup> described from Louisiana a species of *Macrosporium* as the cause of a disease of the foliage. This trouble has also been reported from New Jersey, Connecticut, Rhode Island, and other states. The causal organism was described as follows: "On living leaves of *Daucus carota*, to which it is very injurious. St. Martinsville, La., June, 1888, Langlois, No. 1327. Turning the leaves yellow, then brown, black and killing them entirely. Sterile hyphae erect, at first simple, straight, brown and septate, finally somewhat branched above, and 80 to 100  $\mu$  high by 4 to 6  $\mu$  thick. Conidia clavate, brown, 5 to 7 septate, with one or two of the upper cells longitudinally septate, 55 to 70  $\mu$  by 12 to 14  $\mu$  slender (1- $\frac{1}{2}$  to 2  $\mu$  thick), permanent pedicels 80 to 110  $\mu$  long."

An examination of type material of *Macrosporium carotae*, as well as of fresh material collected in New Jersey and in Washington, D. C., showed that this fungus is specifically distinct from the *Alternaria* species found destructive to the root. Figure 2, F 1-11, shows the conspicuous "pedicel" of the conidia of *Macrosporium carotae* developing on the leaves. It may attain a length of 300  $\mu$  (Fig. 2, F 4), bear one or two branches (Fig. 2, F 4, 7, 8), and show septa varying in number from one to ten (Fig. 2, F. 6); although more usually much shorter, without branches, and provided with but 3 or 4 septa. The description quoted above would seem to indicate that Ellis considered that portion of the spore designated as the pedicel as the lower end. Since the spore is attached to the conidiophore at its obtuse end, this "pedicel" should be considered as an appendage or prolongation of the terminal cell of the conidium.

In size of spore a much greater difference obtains between the fungus on the root and *M. carotae* than Ellis' description might suggest. The large spores of the latter, exclusive of the appendage, may attain a length of 100  $\mu$  or more (Fig. 2, F 1, 2, 4), a width of 30  $\mu$  (Fig. 2, F 1, 2), and show from 9 to 11 transverse septa with 2 or even 3 longitudinal septa further dividing the transverse segments. The cultural characters of the two fungi furnish another easy means of distinguishing them, as *M. carotae* produces, on all the kinds of media on which its cultivation was attempted, only very scant growth, and that consisting largely of colorless submerged mycelium; whereas, as has been pointed out, the fungus causing black rot produces considerable growth, both submerged and aerial, grayish black or bluish black, even on media almost devoid of organic food material.

<sup>1</sup> Ellis, J. B., and Langlois, A. B. New species of Louisiana fungi. *Journal of Mycology* 6: 35-37. Mar., 1890. P. 36. *Macrosporium carotae*, n. sp.

The writers have never observed any catenulate tendency in the fructification of *M. carotae*. According to Elliott<sup>1</sup>, the parasite is to be referred to the genus *Alternaria*, together with other forms possessing spores with a long beak. It is perhaps not impossible to suppose that if the fungus were grown on substrata more suitable than those employed by the writers, a catenulate habit might be manifested. In rather old material, spores may be found showing one or more short processes that have obviously functioned as sporophores in the production of secondary spores (Fig. 2, E 1-5). In nearly every case, however, such proliferation has apparently been associated with the degeneration of the primary spore, as is evidenced by the collapsed condition of many of its segments. It would appear advisable for the present, therefore, to retain the leaf blight fungus in the genus *Macrosporium*. The parasite causing black rot of the carrot is assigned to the genus *Alternaria* and is named *A. radicina* to indicate its relation to the root of its host, to which in nature it seems to be confined.

#### DIAGNOSIS

#### *Alternaria radicina* n. sp.

Aerial mycelium grayish black or bluish black, occurring in extensive mats composed of branching septate hyphae, varying in width from 2.5 to 7.0  $\mu$ ; the wider hyphae more or less distinctly fuliginous, with constrictions associated with the septa; the narrower hyphae more nearly subhyaline, without perceptible constrictions at points of separation. Sporophores forming the terminations of the larger mycelial filaments, or more frequently arising as lateral branches of the latter at irregular but usually rather wide intervals, and at angles usually approaching a right angle; continuous or one to several times septate; fuliginous to dark brown or brown olivaceous, regularly darker than the axial hyphae from which they originate; when lateral, typically 4-5  $\times$  10-25  $\mu$ ; developing successively and in close proximity usually 2-3 primary spores, the points of attachment of which after disarticulation are marked by small dark scars. Spores borne directly on sporophores or more tardily in catenulate arrangement, or singly at apex of short sporophoric processes arising from a spore of the preceding order as attenuated prolongation of terminal segment, or as lateral outgrowth of nonterminal, most frequently of basal, segment; typically straight; clavate, ellipsoid, obovoid, or turbinate; light brown to dark brown or dark olivaceous when fully mature; from 3 to 8 times transversely septate, with a longitudinal segment dividing some or all of the segments not occupying a basal or apical position, and more infrequently

<sup>1</sup> Elliott, John A. Taxonomic characters of the genera *Alternaria* and *Macrosporium*. American Journal of Botany 4: 439-476. Pl. 19, 20. 1917.

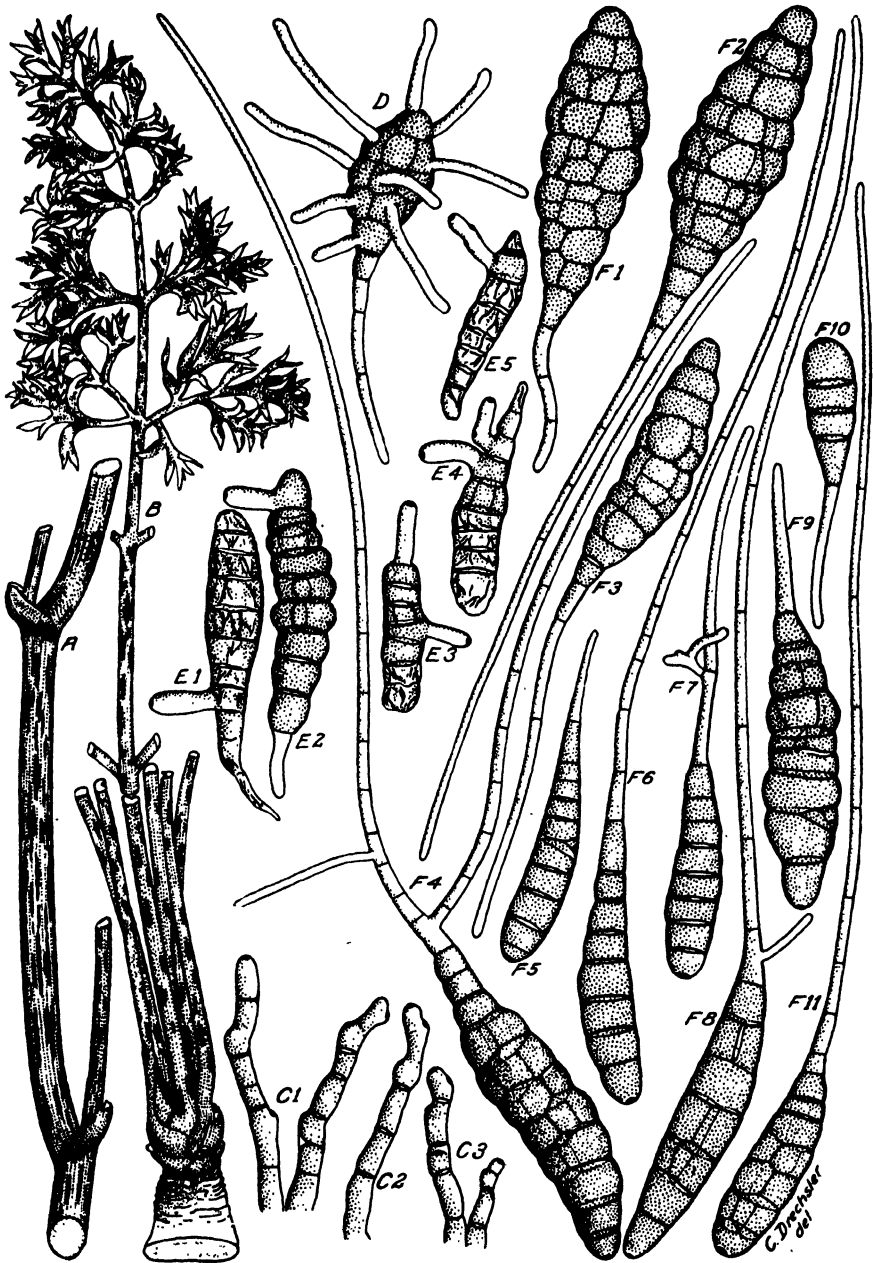


FIG. 2. A. Stem of a mature carrot plant, showing dark longitudinal lesions.  $\times 1$ . B. Petioles and portion of blade of carrot leaf, showing discolored lesions of blight.  $\times 1$ . C. Sporophores, showing variation in size and dark scars marking points of attachment of spores.  $\times 350$ . D. Spore germinating by 9 germ tubes.  $\times 350$ . E. 1-5. Spores more or less collapsed as result of proliferation of sporophoric processes  $\times 350$ . F. 1-11 Spores showing characteristic terminal prolongations variable in length, usually septate; and sometimes branching.  $\times 350$ .



with two longitudinal septa in some of the transverse segments, the septa both transverse and longitudinal associated with constrictions; measuring  $10-22 \times 34-51 \mu$ , the primary spores usually exceeding those of the second or higher order.

Habitat: Found on roots of *Daucus carota*, in Massachusetts, New York, Pennsylvania, and Washington, D. C., causing a moderately destructive storage decay; also as result of inoculation on foliage and stems, where its attack results in conspicuous spotting and in death of leaves.

#### DISTRIBUTION OF THE DISEASE

Observations made during the three seasons that have passed since the disease first came to the writers' attention would indicate that the trouble is generally distributed throughout the carrot storage area of the Eastern United States. Dr. J. I. Lauritzen found the disease on carrots obtained from the Washington market, in April, 1919, approximately the same time that it was found by one of the writers on Long Island. During the seasons of 1919, 1920, and 1921 diseased material has been collected at Peconic, Orient Point, Southold, and Mineola, New York, and at Danvers, South Peabody, Arlington, and Framingham, Massachusetts. In March, 1921, the Pittsburg office of the Bureau of Markets Inspection Service sent in characteristic material that was taken from shipments said to have originated in Pennsylvania.

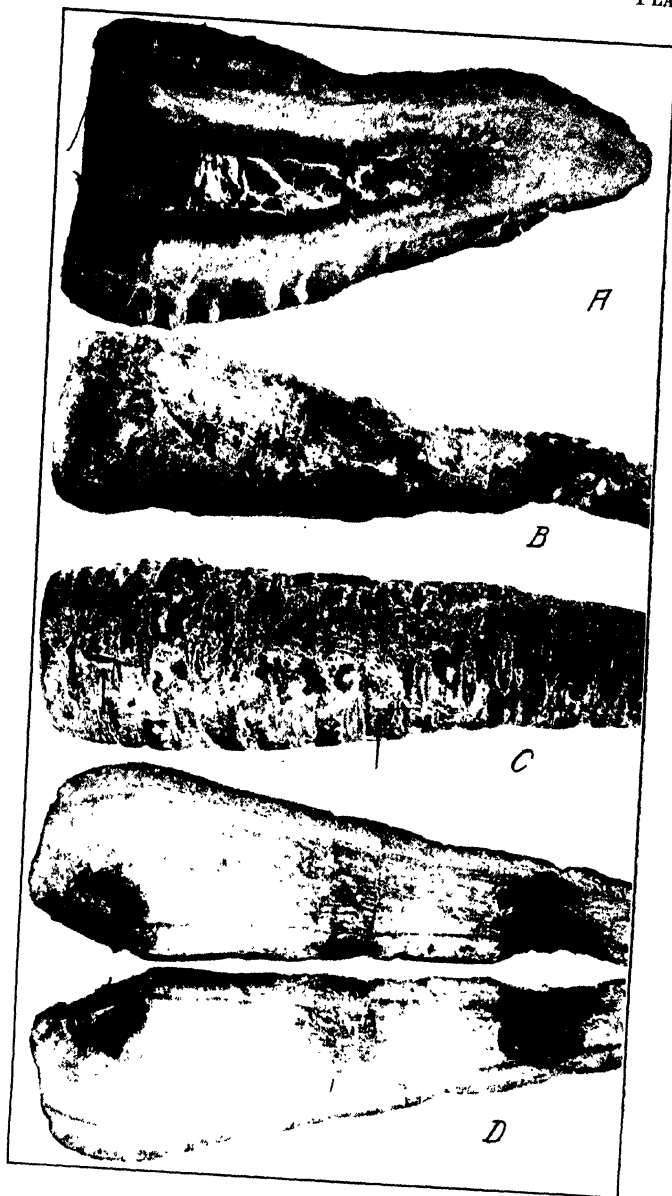
#### SUMMARY.

1. The "black rot" of carrots, a disease which attacks the roots during storage, is caused by *Alternaria radicina* n. sp.

2. Inoculation experiments show that under very favorable weather conditions this fungus may infect the foliage during growth, causing a spotting accompanied by wilting of the affected leaves. This diseased condition has not been found to occur naturally in any of the localities where the "black rot" of the roots developed.

3. The appearance of this disease on the foliage when it occurs as a result of artificial infection is similar to that produced by *Macrosporium carotae*, a distinct species and obligate parasite producing in nature a widely distributed foliage blight of actively growing carrot plants.

4. *Alternaria radicina* may perhaps best be regarded as a facultative parasite of undoubted vigor affecting mature tissues, particularly those of the more or less bruised or wounded roots while being harvested or undergoing storage.



BLACK ROT OF CARROTS

- A. Longitudinal section of a carrot, showing black rot extending into the core of the root.
- B. Three decayed spots resulting from inoculations with *Alternaria radicina*.
- C. One of the carrots which were held as controls, showing two incisions which have healed over.
- D. Longitudinal median section of the inoculated carrot shown in figure B.



# ISARIA ROT OF TOMATO FRUITS

F. J. PRITCHARD and W. S. PORTE

WITH PLATE XII AND ONE FIGURE IN THE TEXT

## INTRODUCTION

A new rot of tomato fruits has occurred in the vicinity of Washington, D. C., during the years 1919, 1920 and 1921. Although nothing further is known regarding its distribution, it would seem from its readiness in attacking tomato fruits to be capable of causing considerable damage. As no reference to it has been found in the botanical literature its chief points of interest are published in this paper.

## DESCRIPTION OF INFECTED FRUITS.

Fruits affected by this rot are usually partly covered by a white cottony surface growth (Pl. XII fig. 7) that later becomes pink or pale orange and granular (Pl., XII, fig. 11). Sometimes the surface filaments are quite coarse (Pl., XII, figs. 9, 10) and occasionally greenish yellow in color, but in a dry atmosphere they may be inconspicuous or even absent (Pl., XII, figs. 12, 4-5). Although the quantity of surface growth varies with the humidity of the atmosphere, the types shown in Pl., XII, figs. 7, 9, 10 and 11 are quite characteristic of this rot.

## CAUSE OF THE ROT.

This rot is caused by a fungus belonging to the genus *Isaria*. Like most species of this genus, it is relatively small and produces very small spores. Although it has been grown on various kinds of culture media and in a wide range of temperatures, it has not developed its perfect stage. It is therefore placed in the group of imperfect fungi.

## PREVALENCE OF THE ROT IN THE FIELD AND IN THE GREENHOUSE.

This rot was first observed on field-grown tomato fruits and has since been found occasionally in the field at the Arlington Experimental Farm. It may be more common in this same area than these observations would seem to indicate, however, for it frequently develops in the interior of the fruits without much disintegration of the tissues and usually appears late upon the surface and may therefore be easily overlooked. It has been found frequently in the experimental greenhouses at Arlington, Virginia, and in Washington, D. C.

## DESCRIPTION OF THE FUNGUS.

*Isaria clonostachoides*, n. sp.

Stroma composed of hyaline agglutinated hyphae (Pl. XII, figs. 13, 14) which become free toward the apex of the fascicle and bear spores at their tips, cylindrical below and conical above, 2–5 mm. high; conidiophores septate, repeatedly divided into whorls of 2 to 4 branches or subulate branchlets; conidia sometimes grouped in heads of the *Acrostalagmus* type but more commonly united into dense angular spikes, at first hyaline but later usually pink or pale orange in mass, 5–8.5  $\mu$  long by 3.2  $\mu$  broad,

Parasitic on tomato fruits in the vicinity of Arlington, Virginia, and Washington, D. C.

## PENETRATION OF THE FRUITS.

Although *Isaria clonostachoides* infects tomato fruits a little more readily through punctures (Pl. XII, figs. 4, 5, 6) than through an unbroken epidermis (Pl., XII, figs 1, 2, 3) it is not dependent on wounds for entrance. It infects both green and ripe fruits (Pl. XII, figs. 7 and 11 respectively) and grows through all the tissues.

Thorough penetration of a green fruit is shown in (Pl. XII, fig. 8) in which the white masses of mycelium may be seen in the seed cavities. It has also spread through the central part, or core, and has even infected the seed, although the infected seed are not visible in the photograph.

## CHARACTER OF THE ROT.

The tissues of tomato fruits infected by *Isaria clonostachoides* remain rather firm especially if green. Such an infected fruit as shown in plate XII, figure 8, for instance, seems quite tough when cut or squeezed. The rot causes very little odor and what little is produced is not unpleasant.

## EFFECT OF INOCULATING TOMATO FRUITS.

One hundred green and one hundred ripe tomato fruits, half of which were pricked and half unpricked, were inoculated with *Isaria clonostachoides* in six series of experiments and kept in moist chambers at 24° to 25° C. The air in the chambers was kept moist by spraying the fruits with distilled water whenever their surfaces began to dry. The percentage of infections obtained on pricked green fruit was 60; on unpricked green fruit 40; on pricked ripe fruit 90; and on unpricked ripe fruit 50. An equal number of control fruits remained free from infection.

Two sets of inoculation experiments were made on fruits grown in the greenhouse. Both green and ripe fruits some of which were pricked and some unpricked were inoculated with a pure culture of this fungus

and covered at the point of inoculation with a small piece of wet cotton. Seventy-five fruits were thus inoculated and as many more were used as checks. Due probably to the drying of the cotton, none of the unpricked fruits showed any symptoms of the rot. About 50 per cent. of the pricked green fruits became infected but showed little surface growth while 60 per cent. of the ripe fruits developed the characteristic symptoms of the rot including the growth of mycelium on the surface.

Two hundred small green to large green tomato fruits were inoculated with *Isaria clonostachoides* and placed in a moist atmosphere in constant temperature chambers ranging from 0° to 40° C. The unpricked as well as the pricked fruits became infected but the ripening fruits and small green fruits were more readily infected and developed more rot than the large green fruits. Fully 70 per cent. of the fruits became infected in the chambers kept at about 29° C.

The seed of tomatoes infected by this fungus frequently become brown to dark brown in color and when transferred under aseptic conditions to sterile plates of agar-agar often become covered with a pure culture of the fungus.

#### EFFECT OF INOCULATING TOMATO PLANTS.

Twenty young tomato plants were inoculated in the roots and 20 were inoculated in the stems with *Isaria clonostachoides* and kept in a moist chamber 48 to 60 hours but no infections developed on the roots and only a few minute brown spots appeared on the stems and these made no further development. Millions of spores sprayed on the leaves of tomato plants growing in a relatively moist greenhouse caused no infections. This fungus would therefore seem to have very little parasitic action on the tomato plant.

#### GROWTH OF ARTIFICIAL CULTURE MEDIA

*Isaria clonostachoides* grows fairly rapidly on glucose agar, potato agar, potato plugs, lima bean agar, oatmeal agar, beef agar, and string bean agar but very slowly on corn meal agar. It imparts a greenish yellow color to oatmeal agar but produces no such change in any of the other media mentioned.

A typical 14-day old colony grown on string bean agar is shown in plate XII, figure 12. Erect filaments similar to those in the center of the colony usually appear on the entire surface when it becomes older. On a somewhat drier culture medium, as in an older plate; on the surface of tomato fruits which have become rather tough and dry from the action of the fungus; and on cotton and sweet clover stems the erect spore-bearing fascicles are so dense that the individual filaments composing them cannot usually be traced. Under these conditions the fruiting synnema is larger and denser than those shown in plate XII,

figures 12 and 14 but bears the same type of conidiophores on its apical and lateral surfaces.

The colonies are at first white but usually become pink or pale orange during spore production.

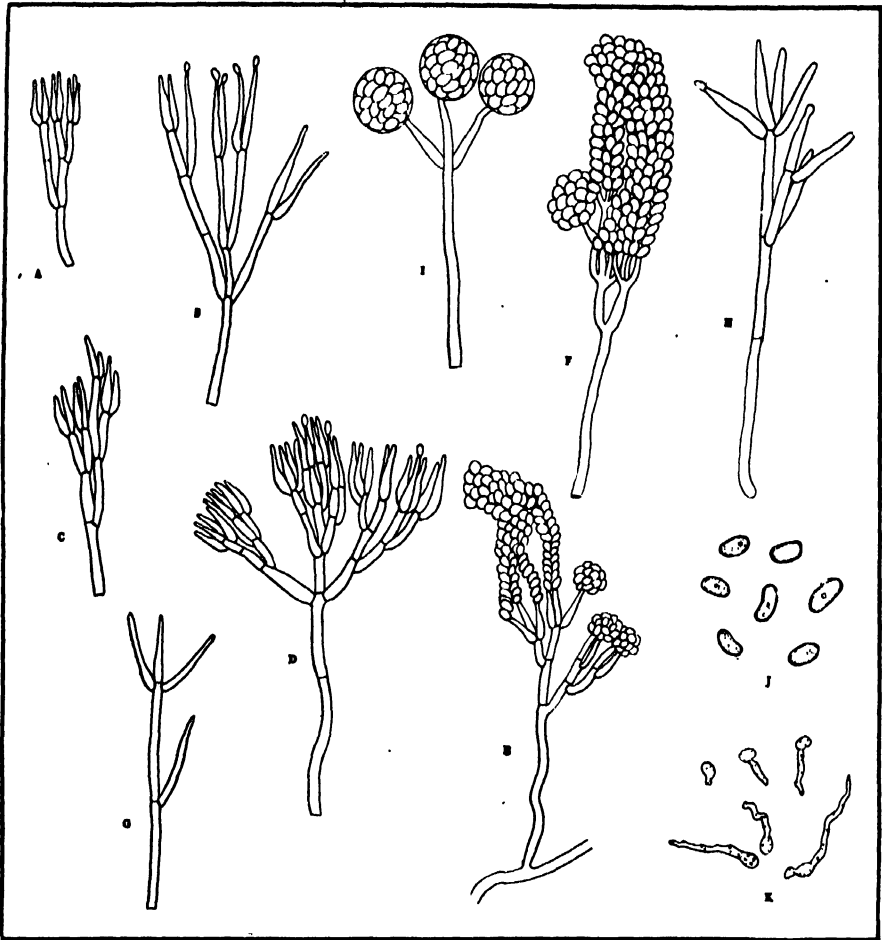


FIG. 1. Drawings of *Isaria clonostachoides*, n. sp.  $\times 400$ , J  $\times 880$ ; A-D, Conidiophores of the compact type; E-F, Parts of conidiophores showing the structure of the conidial spikes; G-H, Conidiophores of the open, sparingly branched type; I, An open type of conidiophore bearing globular masses of conidia surrounded by a film of mucus; J, Conidia highly magnified; K, Germinating conidia.

#### RELATION OF TEMPERATURE TO INFECTION

The optimum and limiting temperatures for growth and infection by this fungus are recorded in table 1.

TABLE I

*Influence of temperature on growth and infection by Isaria clonostachoides*

Temperature relations	Temperatures degrees C <sup>1</sup>		
	Minimum	Optimum	Maximum
Growth on culture media.....	7	29	36
Infection of tomato fruits.....	14	29	32
Germination of spores.....	7	31.5	36

The optimum temperature is apparently the same for growth on artificial culture media as for infection of tomato fruits. The maximum and minimum temperatures for growth on culture media, however, are higher and lower respectively than for infection.

## MORPHOLOGY OF THE FUNGUS

The hyphae of *Isaria clonostachoides* are grouped in bundles which grow more or less horizontally but become erect toward the tips causing a coarse filamentous appearance (Pl. XII, figs. 12, 13, 14). The bundles are cylindrical for the greater part of their length but, owing to the gradual separation and divergence of the ends of the hyphae, especially toward the apices, the tips taper to a point and not infrequently look to the unaided eye like repeatedly split hairs (edge of colony Pl. XII, fig. 14). When the tips of the bundles produce conidiophores and spores, however, they have an elongated conical appearance (Pl. XII, figs. 13, 14).

There are two types of conidiophores,—one dense or compact, and the other open, stiff, and more sparingly branched. In the dense type (Figs. 1 A, 1B, 1C,) the branches and branchlets are very narrowly separated and the conidia, one row from each branchlet, cohere laterally, forming a dense, angular spike (Figs. 1E, 1F) like those of the genus *Clonostachys*. A conidiophore of this type often bears several spikes, one from each terminal whorl or dense group of whorls of branchlets. In the other type of conidiophore (Figs. G1, 1H, 1I,) in which the branchlets are fewer in number and more widely separated, the conidia form singly on the ends of the branchlets as in the more dense type but usually cohere in globular aggregations surrounded by a film of mucus. This type of conidiophore does not seem to pass over into the more dense type although occasionally a few globular masses of conidia are found on the dense type (Figs. 1E, 1F).

The conidia on the two types of conidiophores are alike in size, shape, and other appearances (Figs. 1J, 1K). They are oval to curved in form and not infrequently somewhat blunt at one end, usually being rounded a little more at one corner than at the other. When produced on the more compact type of conidiophore they come into lateral contact with other conidia from the same whorl of branchlets and are carried

<sup>1</sup> These temperatures are only approximately correct as there was a difference of 3 or 4 degrees between some of the adjoining chambers.



outward in mass by the successive production of conidia below them, giving rise to a spike. When a branchlet is somewhat separated from the other branchlets of a whorl, (Figs. 1E, and 1F,) its conidia do not make this lateral contact with conidia from another branchlet and therefore form a globular mass instead of a spike. Apparently this is the only reason that the conidia on the compact conidiophores form spikes while those on the open less branched conidiophores form heads of the *Acrostalagmus* type. To form either spikes or heads however the conidia must possess an adhesive surface.

It is apparent from the foregoing description of this fungus that it is similar in some respects to *Verticillium*, *Acrostalagmus*, and *Clonostachys*. Those who place very little emphasis on the genetic significance of the synnema may regard it as one of these genera, especially *Clonostachys*. In fact, in the absence of the perfect stage, its genetic relationship is questionable. However, as the synnema is used as a family character in the current systems of classification and serves to separate the *Moniliaceae* from the *Stilbaceae*, this fungus would seem to belong to the genus *Isaria* of the latter group. As it does not fit the description of any of the species of *Isaria* already described it has been designated, at least tentatively as *Isaria clonostachoides*.<sup>1</sup>

#### SUMMARY

A new rot of tomato fruits caused by *Isaria clonostachoides*, n. sp. has been observed quite frequently in the vicinity of Washington, D. C., during the years 1919, 1920 and 1921. As the fungus produces innumerable spores and infects both green and ripe fruits readily, it would seem capable of causing considerable damage.

#### DESCRIPTION OF PLATE XII

*Isaria* rot of tomato fruits and appearance of the colonies on artificial culture media.

Figs. 1-3. Fruits infected through an uninjured epidermis.

Figs. 4-6. Fruits infected through needle punctures.

FIG. 7. Infected green fruit showing a white cottony surface growth.

FIG. 8. Green fruit showing white masses of the fungus in the seed cavities and core.

Figs. 9-10. Fruits showing bundles, or fascicles, of hyphae on the surface.

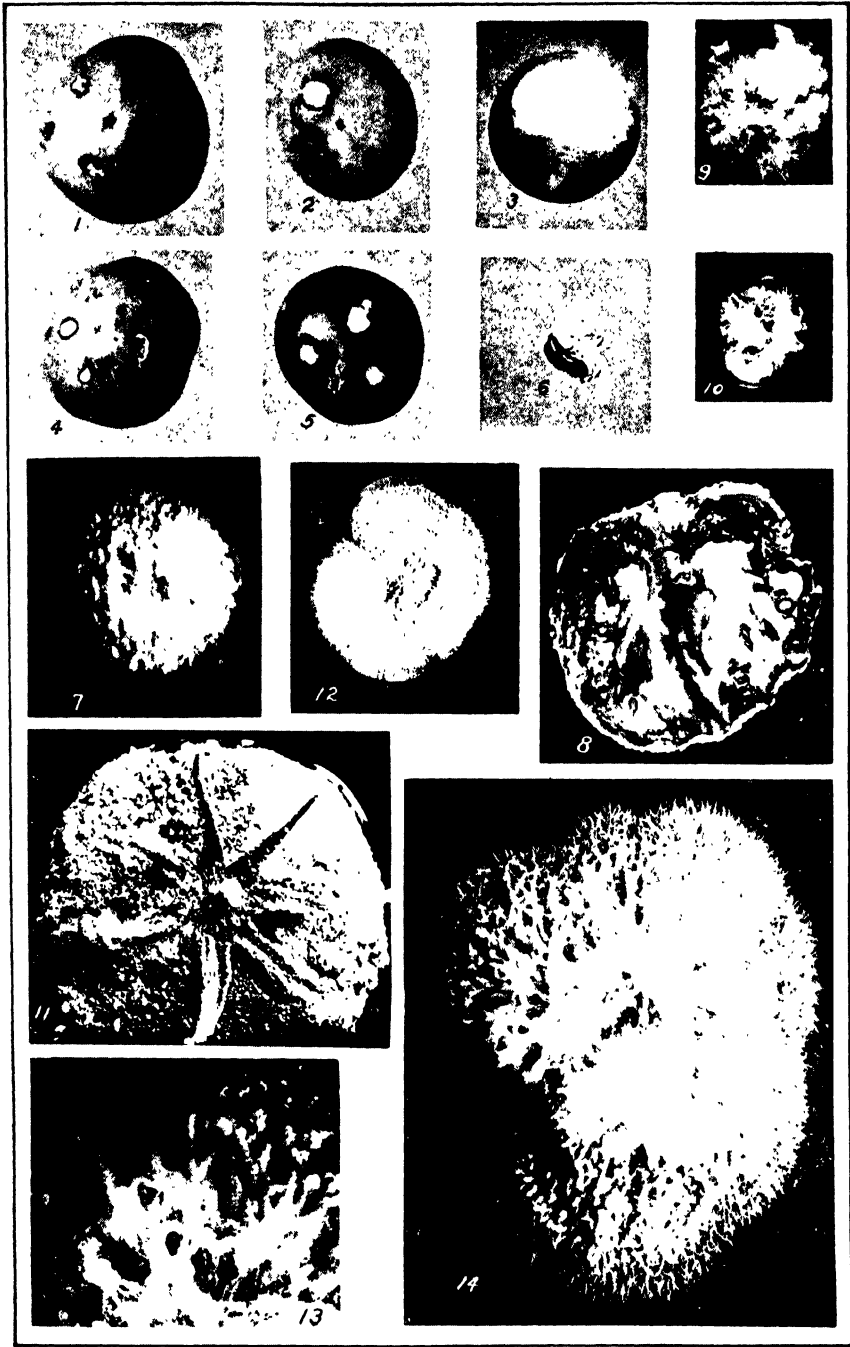
FIG. 11. A ripe tomato fruit covered by a pink or pale orange granular growth of the fungus.

FIG. 12. A typical 14-day old colony on string bean agar.

FIG. 13. Stromata highly magnified.  $\times 25\frac{1}{4}$ .

FIG. 14. Colony of the fungus showing fascicles and stromata.  $\times 6\frac{1}{4}$ .

<sup>1</sup> *Isaria filamentosa* Sacc. Mich. I, p. 132, F. ital. T 840 has been found growing on putrid stems of *Solanum* but the lateral diameter of its spores varies from 2.5 to 3.5  $\mu$  while that of *Isaria clonostachoides* varies very little from 3.2  $\mu$ . Moreover, *Isaria filamentosa* is white and does not form spore-heads or spore-ears.



ISARIA ROT OF TOMATO FRUIT



# EFFECT OF DELAYED PLANTING ON GERMINATION OF SEED WHEAT TREATED WITH FORMALIN<sup>1</sup>

HARRY BRAUN

WITH PLATE XIII AND THREE FIGURES IN THE TEXT

It is often necessary to hold formalin-treated seeds for several days or a week, when immediate sowing after treatment is prevented by heavy rains and unworkable ground. Numerous cases of severe injury to germination, noted among others by Coons<sup>2</sup>, have resulted from storage of seed wheat treated by the concentrated formalin dry method or even by the sprinkling or wet method. The present paper is a report of a small part only of a long series of experiments made to determine the effect of delayed planting, on the germination of seed wheat treated with formalin by the ordinary method and by the presoak method recently outlined by the writer<sup>3</sup>.

## EXPERIMENTAL METHODS

In December, 1921, five standard varieties of wheat were used: Marquis, Fultz, Turkey, Purple Straw, and Fulcaster. One set of seeds of each variety, in quart lots, was dipped for half an hour in a formaldehyde solution of 1 part commercial formalin to 320 parts of water, drained and covered one hour. Another set was treated in the same way, except that the seeds were first soaked in water 10 minutes, drained and covered five and a half hours to permit absorption of the water vapor by the seeds, before receiving the formaldehyde treatment. All seeds were then spread out to dry over night and part of the next day, in a layer about an inch thick, with occasional stirring for aeration. At the end of 24 hours 300 seeds of each variety were planted in the greenhouse in triplicate pots of 100 seeds each, with triplicate pots of untreated checks. The remainder of the treated seeds was placed in bags and kept a week in the laboratory (room temperature averaging 23° C.) then 300 seeds of each variety were counted out as before and planted in

<sup>1</sup> The author wishes to acknowledge his indebtedness to Dr. Erwin F. Smith for criticism and advice throughout this investigation.

<sup>2</sup> Coons, G. H. The Use of Formaldehyde to Control Cereal Smuts. *In Mich. Agr. Exp. Sta. Quart. Bul.*, v. I, no. 1, p. 11-14. 1918.

<sup>3</sup> BRAUN, HARRY. Presoak Method of Seed Treatment: A Means of Preventing Seed Injury due to Chemical Disinfectants, and of Increasing Germicidal Efficiency *In Jour. Agr. Res.* 19: 363-392. 1920.

the greenhouse in the same manner as those planted one day after treatment and with as many untreated checks.

Counts of germination of each planted set were made on the seventh, ninth and tenth days after planting.

On its completion the experiment was repeated (January, 1922) using the same procedure throughout. The germination percentages for the December and January plantings are charted in figures 1 and 2, and correspond closely to previous experiments in which these and other varieties were used.

#### DISCUSSION

The different appearance of the seedlings of the delayed January plantings, where 1, 2 are the controls, 3, 4 the ordinary formalin treatment, and 5, 6 the presoak formalin treatment, is shown for 4 of the 5 varieties on plate XIII.

A comparison of the data for the checks (A, AA) and the formalin-treated<sup>1</sup> seeds, planted one day (B) or one week after treatment (BB), shows in all cases definite and considerable injury to germination due to the treatment. Delay in germination is striking in that only *one-third to one-half* of the seedlings that developed from treated seeds appeared by the seventh day (solid bar), whereas the *majority* of the untreated seeds had germinated by this time. In Marquis and Fulcaster (first experiment) the final germination of formalin-treated seeds kept one week show an increase of 4 to 8 per cent over formalin-treated seeds kept one day, though marked retardation is evident in Fulcaster (solid bar). In all other cases there is retardation and a decreased germination of 3 to 22 per cent in formalin-treated seeds after a week's storage, as compared with those held one day. Averages of all varieties (lowest graphs) indicate clearly the general retardation and marked cumulative injury caused by storage of the formalin-treated seeds.

A comparison of the data for presoaked formalin-treated seeds, planted one day (C) or one week after treatment (CC), with corresponding checks (A, AA), and with formalin-treated seeds (B, BB), gives for the former: (1) in all cases, better germination than ordinary formalin-treated seeds; (2) germination equal to or better than that of corresponding checks, in all cases, except three where a decrease of 1 to 4 per cent as compared with the checks is not beyond the limits of experimental error; (3) marked stimulation of germination, since most of the seedlings appeared before the 7th day (solid bar). The averages

<sup>1</sup> The term "formalin-treated" will be used throughout to denote seeds dipped in formalin 1:320 half an hour, then drained and covered one hour; "presoaked formalin-treated" to denote seeds treated in the same manner but preceded by a ten-minute dip in water and a 5½ hour water-vapor absorption period.

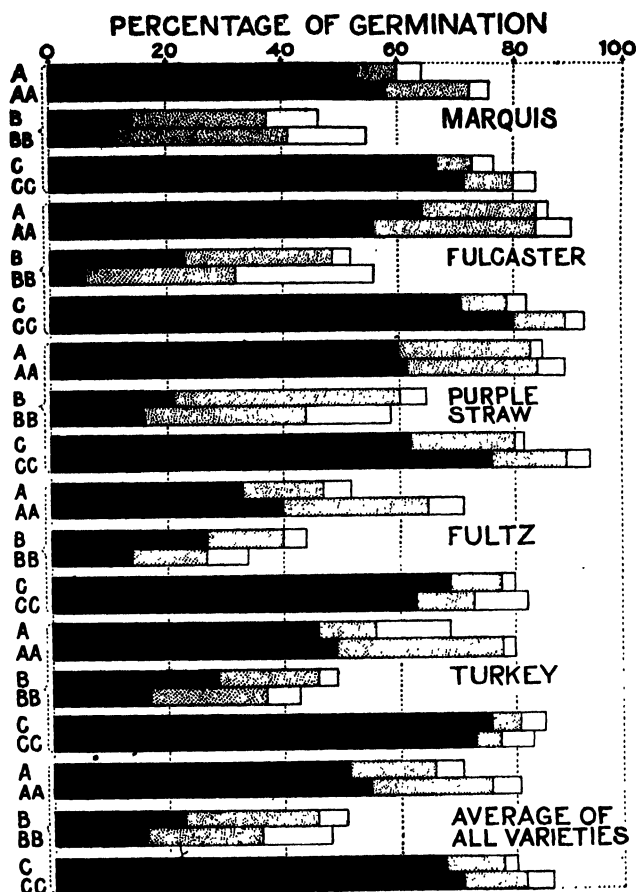


FIG. 1. Comparison of common *vs.* pre-soak formalin treatment of seed wheat and effect of immediate planting *vs.* holding treated seed one week before planting. Germinations on 7th day after planting, solid bar; 9th day, shaded bar; 10th day, open bar.

A—Check, untreated. Planted Dec. 3.

AA—Check, untreated. Planted Dec. 9.

B—Seeds soaked in  $\text{CH}_2\text{O}$ , 1:320 half-hour, (Dec. 2), drained, covered one hour, dried overnight in one-inch layer, planted Dec. 3.

BB—Same set as B, except that seeds were kept in bags one week and planted Dec. 9.

C—Seeds soaked in water ten minutes (Dec. 2), drained, covered  $5\frac{1}{2}$  hours, then treated with  $\text{CH}_2\text{O}$  as detailed in B, dried overnight in one-inch layer and planted Dec. 3.

CC—Same set as C, except that seeds were kept in bags one week and planted Dec. 9.

for all varieties indicate a distinct gain by use of the presoak method whether the seeds were held one day or one week before planting.

The constant higher germination of checks (see averages) in the plantings of December 9 (AA) and of January 4 (A), as compared with those of December 2 (A) and January 11 (AA), was found to be correlated with variations in amount of sunshine, which was more abundant during the germinating periods of these plantings than during the corresponding periods of the other plantings. Other factors, soil, watering and depth of planting, were constant and under control. Prevalence of sunlight or cloudiness may be expected to influence germination, by affecting soil temperature. Since, however, all seeds of a planted set, whether treated or untreated, are influenced equally by a factor of this nature, this factor may be stabilized and the data for the various plantings co-ordinated by transposing absolute to relative germination; *i. e.*, by considering final germinations of controls as 100 per cent and calculating germinations of treated seeds in reference to this norm. A clearer picture of the proportional effects of treatments, relative to a normal germination, may thus be obtained. Data thus calculated from averages in all plantings is charted in figure 3.

Figure 3 shows clearly: (1) delay and decrease in germination of formalin-treated seeds dried and planted a day after treatment (B); (2) accentuated injury to seeds similarly treated and kept a week before planting (BB); (3) no appreciable injury and some stimulation in presoaked formalin-treated seeds dried and planted a day after treatment (C) also evident throughout experiments reported in a previous paper<sup>1</sup>; (4) for presoaked formalin-treated seeds kept a week (CC), a slight decrease in germination as compared with treated seeds kept only a day but no appreciable injury as compared with checks, and germinating nearly twice as well as non-pres soaked formalin-treated seeds kept a week.

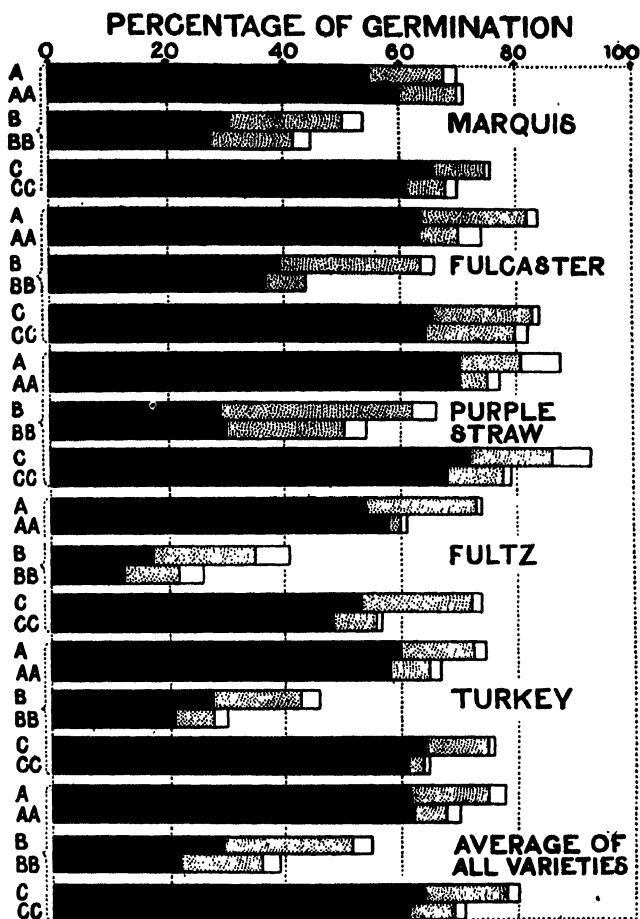
On the whole it may be concluded that, whereas cumulative injury is apparent in formalin-treated wheat seeds held a week before planting, no appreciable injury is shown after a week's storage by seeds treated with formalin in the same way, but preceded by a ten-minute dip in water and a 5-½ hour water-vapor absorption period.

Full discussion of the causes involved is reserved for a later paper, but the following points may be briefly indicated. The well-known hardening effect of formalin, in this case on the pericarp, was considered by McAlpine<sup>2</sup> and G. P. Darnell-Smith and Carne<sup>3</sup> as a contributing cause

<sup>1</sup> Braun, Harry. *l. c.*

<sup>2</sup> McAlpine, D. Effect of formalin and bluestone on the germination of seed wheat. *In Agr. Gaz. N. S. Wales*, 17: 423-439. 1906.

<sup>3</sup> Darnell-Smith, G. P., and Carne, W. M. The effect of formalin on the germination of plants. *In 3d Rpt. Govt. Bur. Microbiol. (N. S. Wales) 1912*, p. 178-180. 1914.



g. 2. Result of January treatments and plantings. Same terminology as in Fig. 1. A, B, C planted January 5; AA, BB, CC planted January 11.

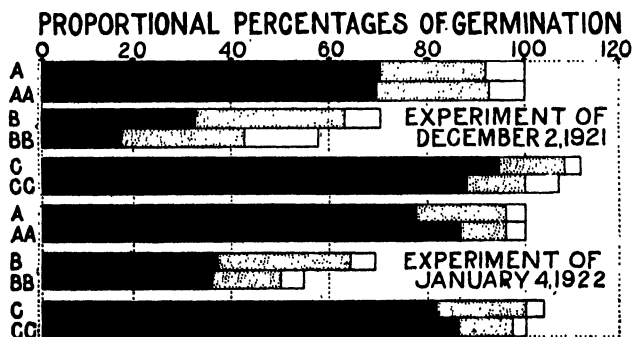


FIG. 3. Data of figures 1 and 2 but recalculated, as explained in the text, the checks being taken as 100 per cent.



to cumulative storage injury after formalin treatment, in that a hardened pericarp prevents the primary root from pushing through or delays it. It is evident that the absorption of water-vapor by seeds previous to formalin treatment, tends to soften the tissue and counterbalance this hardening effect, and also tends to dilute the full-strength formalin absorbed by the pericarp and consequently to decrease its hardening and killing power.

Another possible cause of cumulative storage injury is the persistence of paraformaldehyde on the surface of treated seeds, suggested by G. P. Darnell-Smith and Carne<sup>1</sup>, Coons<sup>2</sup> and Miss Hurd<sup>3</sup>, who obtained typical formaldehyde reactions on dry-stored, formalin-treated seed wheat with Tollen's reagent (silver nitrate-sodium hydroxide-ammonia). Through absorption during the treatment period, the formaldehyde is probably also persistent, either in the polymer form or in a highly concentrated solution, within the pericarp tissues of the stored seeds as well as on the surface.

Tests with Tollen's reagent<sup>4</sup> on formalin-treated seeds dried and kept one week, and on presoaked formalin-treated seeds dried and kept one week, showed for the latter a formaldehyde reaction approximately one-fourth as intense as the former (See test tubes Pl. XIII, 1A). Similar tests made one day after treatment and drying showed a formaldehyde reaction half as intense for presoaked formalin-treated seeds as for ordinary formalin-treated seeds (Pl. XIII, 1B).

These differences in amount of persistent formaldehyde (or the polymer, since Tollen's test does not distinguish between the two) indicate that less formaldehyde persists on presoaked formalin-treated seeds, through (1) greater evaporation of formaldehyde from the increased surfaces of the swollen seeds during the aeration period (2) less tendency for the polymer to form on the surface during the subsequent storage period, in the presence of the moist tissues immediately beneath; and as a corollary to this, absorption of surface formaldehyde during the storage period and its dilution beyond the point of injury by the water previously absorbed by the seeds.

<sup>1</sup> Darnell-Smith, G. P., and Carne, W. M. l. c.

<sup>2</sup> Coons, G. H. l. c.

<sup>3</sup> Hurd, A. M. Injury to seed wheat resulting from drying after disinfection with formaldehyde. *In Jour. Agric. Res.* 20: 209-244. 1920.

<sup>4</sup> Equal parts of 10 per cent aqueous solutions of silver nitrate and of sodium hydroxide are mixed together and the resulting precipitate dissolved by gradual addition of strong ammonia. On adding 1 c.c. of the clear liquid to a solution containing formaldehyde, the solution slowly turns to a deep brown, and a black precipitate is finally formed. The rate and depth of coloration, and the amount of precipitate indicate the relative amount of formaldehyde present.

## SUMMARY

(1) The hardening effect of persistent formaldehyde or its polymer on the pericarp is counteracted in the presoak method by the softening of seed tissues through previous absorption of water-vapor.

(2) Formaldehyde or its polymer does not persist on presoaked formalin-treated seeds to the same degree as on formalin-treated seeds without presoaking, either through increased evaporation during the aeration period, or less tendency to polymerize in the presence of water in the pericarp tissues during the storage period, or through absorption and dilution in the presoaked seed tissues.

(3) Experiments herein reported indicate that presoaked formalin-treated seed wheat may be held several days or a week before planting, without appreciable injury to germination.

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## DESCRIPTION OF PLATE XIII

PLATE XIII (Seedlings).—Effect of delayed planting on germination of seed wheat treated with formalin with and without presoaking; photographed ten days after planting. Experiment of January 4, 1922.

1, 2, controls, 100 seeds planted in each pot; 3, 4, seeds soaked in formalin 1:320 half hour, drained and covered one hour, dried overnight in one-inch layer, kept in bags one week, then planted, 100 seeds to each pot; 5, 6, seeds soaked in water 10 minutes, drained and covered  $5\frac{1}{2}$  hours, then treated with formalin as in 3, 4, dried overnight in one-inch layer, kept in bags one week, then planted, 100 seeds to each pot.

FIG. A. Turkey: 1, 2, controls, 67 per cent germination; 3, 4, formalin-treated, kept one week, 30 per cent germination; 5, 6, presoaked formalin-treated, kept one week, 65 per cent germination.

FIG. B. Purple Straw: 1, 2, controls, 77 per cent germination; 3, 4, formalin-treated, kept one week, 54 per cent germination; 5, 6, presoaked formalin-treated, kept one week, 79 per cent germination.

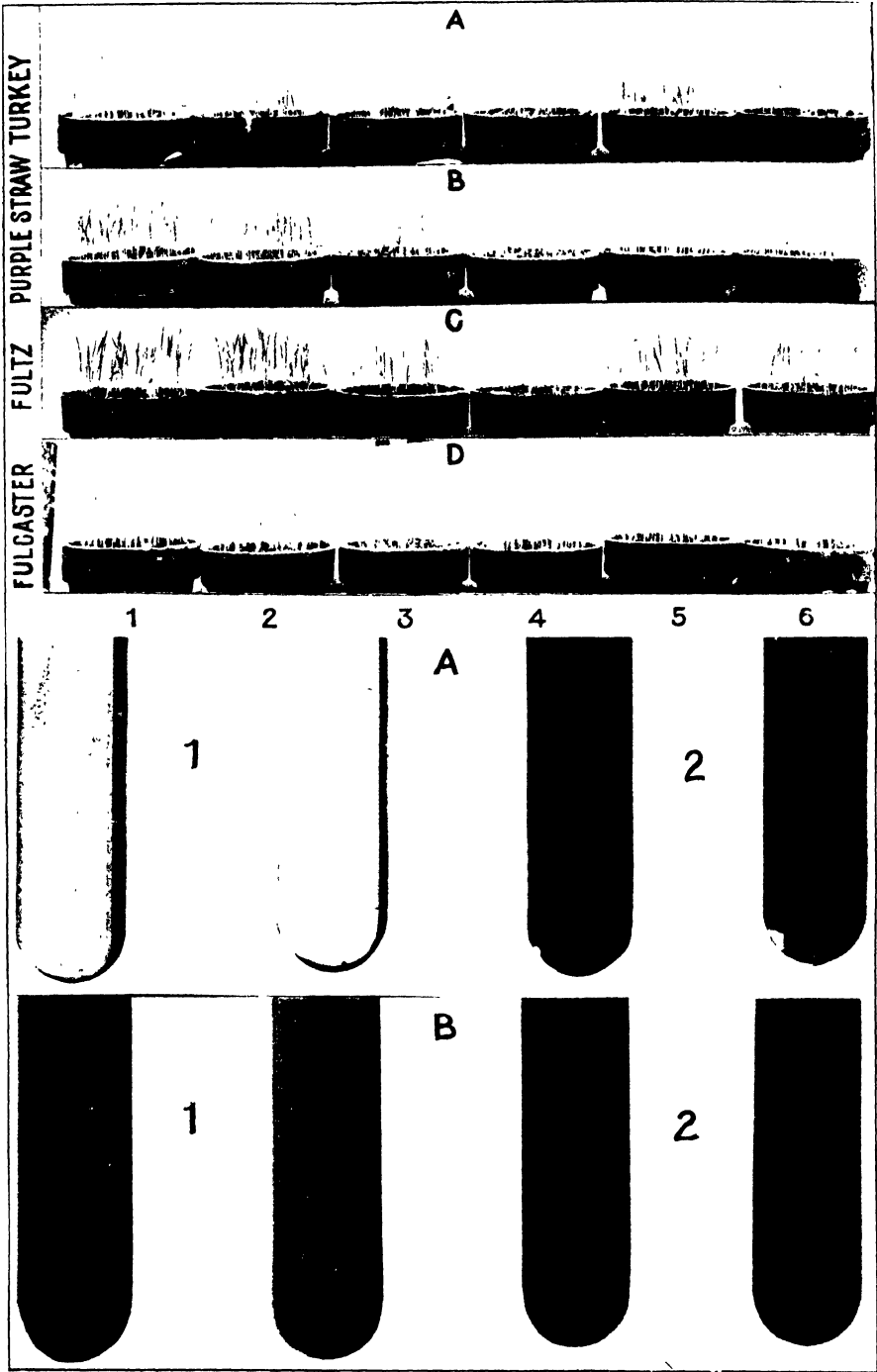
FIG. C. Fultz: 1, 2, controls, 61 per cent germination; 3, 4, formalin-treated, kept one week, 26 per cent germination; 5, 6, presoaked formalin-treated, kept one week, 58 per cent germination.

FIG. D. Fulcaster: 1, 2, controls, 74 per cent germination; 3, 4, formalin-treated, kept one week, 44 per cent germination; 5, 6, presoaked formalin-treated, kept one week, 82 per cent germination.

PLATE XIII (Tubes).—Relative persistence of formaldehyde on seed wheat treated with formalin, with and without presoaking.

FIG. A. Fifteen-gram samples from treated seeds, kept one week in bags, were shaken up with 25 c.c. of distilled water for 5 minutes; 10 c.c. samples pipetted into tubes, then one c.c. Tollen's reagent added to each in rapid succession; tubes photographed 10 minutes after addition of reagent. (1) Washings from presoaked formalin-treated seeds; (2) washings from formalin-treated seeds, without presoaking. The reaction of (2) is approximately 4 times as intense as that of (1), indicating the relative amounts of persistent formaldehyde after a week.

FIG. B. Similar tests made one day after treatment. (1) Washings from presoaked formalin-treated seeds; (2) washings from formalin-treated seeds, without presoaking. The reaction of (2) is approximately twice as intense as that of (1).



GERMINATION OF SEED WHEAT



NOTES ON THE ETIOLOGY AND SPECIFICITY OF THE  
POTATO TIP BURN PRODUCED BY EMPOASCA  
MALI LE BARON.<sup>1</sup>

JOHN R. EYER

WITH PLATE XIV AND ONE FIGURE IN THE TEXT

Recent investigations made by plant pathologists and entomologists to determine the etiology of potato tip burn have led to the conclusion that this disease is either of a physiological nature or caused by the feeding of the potato leafhopper (*Empoasca mali* LeB.). Lutman,<sup>2</sup> writing on the physiological aspect of the disease, has advanced the theory that the burning is due to excessive heat and sunshine during the hot portions of the growing season. He has also noted that the inordinate transpiration from the portions of the leaflets directly affected results in a wilting which he has attributed to the relation existing between the leaf transpiration and the water pores or hydathodes situated near the tip and along the marginal vein.

The association of a potato tip burn with the potato leafhopper was first observed by Ball<sup>3</sup> in 1918 who demonstrated a definite relation between the leafhopper and this disease. This burning differed from the physiological form by its initial appearance on the midrib and veinlets of the leaflets followed by the wilting of the interstitial tissue. Ball concluded that the explanation of the disease was to be found in "some specific transmitted by the insect" which when introduced into the circulation thru the veins caused the death of the tissue supplied by them.

To determine the nature and transmissibility of the "specific" injected into the potato by this species of leafhopper, inocula prepared by macerating the nymphal and adult stages of the insect in sterile water or 10-70 per cent alcohol, were introduced into the leaves of growing potato plants. A small hypodermic needle or capillary pipette was used and the inoculum was forced well into the tissue thru the midrib of the leaflet. Other plants were inoculated in a like manner with

<sup>1</sup> Contribution from the Department of Botany, The Pennsylvania State College. No. 36.

<sup>2</sup> Lutman, B. F. Tip burn of the potato and other plants. Vt. Agr. Exp. Sta. Bul. 214, 1919.

<sup>3</sup> Ball, E. D. The potato leafhopper and the hopperburn that it causes. Wisconsin Dept. of Agr., Bul. 23, 1919.

sterile water or alcohol to serve as checks. Inoculated plants were placed in the direct sunlight both in the greenhouse and in the field to contrast the effect produced under glass with that in the open and all were carefully screened from attack by leafhoppers.

In the field the plants evidenced a wilting and chlorosis of the tips of the leaflets eight days after inoculation and developed a characteristic tip burn in twenty days. In the greenhouse the progress of the disease was slower, the first symptoms appearing in thirteen days followed by the browning of the tips and lateral margins twenty-eight days after inoculation. The production of the disease artificially consumed more time than when it was naturally caused by the feeding of the leafhoppers in the field. In this case the initial symptoms were noticeable twenty-four hours after the insects began feeding and severe tip burn was produced in about four days by the nymphal stage of the leafhopper. (See Pl. XIV, fig. 2). Petiole inoculations usually resulted in chlorosis, browning, and death of the entire leaf. All check plants remained perfectly healthy, both in the greenhouse and in the field.

Inocula prepared from the leafhopper nymphs produced tip burn sooner and more readily than did those extracted from the adults. This was in confirmation with the results of Fenton<sup>1</sup> who demonstrated, by a series of cage experiments, that most of the foliage burning is caused by the nymphal stage of the insect.

Sterile water, or alcohol varying in strength from ten to seventy per cent, was used in preparing the inocula and the virulence of the extract was in direct proportion to the number of leafhoppers macerated in the solution and was in no way affected by the amount of alcohol.

Attempts to produce tip burn by pricking the inoculum into the interstitial leaf-membrane or by rubbing it on the healthy leaf were unsuccessful; the disease was only produced by direct inoculation of the leafhopper extract into the vascular system of the plant, either thru the midrib, veinlets, or stem. Mechanical injury of the midrib sometimes resulted in a browning similar to tip burn but this condition was restricted and did not spread beyond the group of cells injured.

To ascertain whether the causative principle of the disease existed in the injured tissue after infection by leafhoppers an alcoholic extract was made from leaves showing varying stages of hopperburn. This was then inoculated into the midribs of healthy leaflets and the disease was produced six weeks after inoculations; a somewhat longer period than in the case of the inoculum prepared directly from the leafhoppers. These symptoms compared closely with the disease produced by the leafhopper extract and were just as marked.

<sup>1</sup> Fenton, F. A. Progress report on the season's work on the production of potato tipburn. Jour. Econ. Ent., 4: 71-79, 1921.

The specificity of the disease transmitted to the potato plant by *E. mali* was demonstrated by a series of comparative inoculations with inocula prepared from a number of sucking insects which normally feed on the potato plant. Extracts prepared from the potato aphid (*Macrosiphum solanifolii* Ashm.), the tarnished plant bug (*Lygus pratensis* Linn.), and the apple leafhopper (*Empoasca unicolor* Gill.), produced negative results. Inoculations of the false chinch-bug (*Nysius ericae* Schill.) caused chlorosis and death of the leaflets but this condition did not resemble tip burn.



Fig. 1. Tipburned potato plant sixteen days after inoculation with leafhopper extract. Exposed to the direct rays of the sun in the green house.

A relation between the amount of light and the progress of the disease was noted in all of these experiments. (See Pl. XIV, figs. 1, 2). The initial appearance and the development of the burning was much slower in plants grown in the greenhouse in winter than in those grown in the field during the summer months. (See Fig. 1). Cages of heavy cheese cloth which shaded the plants retarded the development of the disease. (See Pl. XIV, Fig. 1.) It was found possible however, to produce



tip burn in the absence of direct sunlight either thru the feeding of the leafhoppers or by inoculating the plants. In this case the development of the burning required four or five weeks and was superseded by an extensive chlorosis of the leaflets.

#### CONCLUSIONS

A tip burn of the potato plant is produced by the extract made from macerated leafhoppers of the species *Empoasca mali* LeB. and is transmissible by direct inoculation.

The active principle of this extract is most virulent in the nymphal stage of the leafhopper.

The "specific" of tipburn is present in diseased leaf-tissue after infection by the leafhopper and may be transmitted to healthy plants by re-inoculation.

This substance is specific and the disease has not been simulated by inoculation with extracts from or by the direct feeding of insects other than *E. mali*, or by mechanical injury.

Sunlight is an important factor in the progress of tipburn after its inception but the absence of sunlight does not prevent the disease.

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#### OBSERVATIONS ON FROST PROTECTION AND DROUTH SPOT OF APPLE

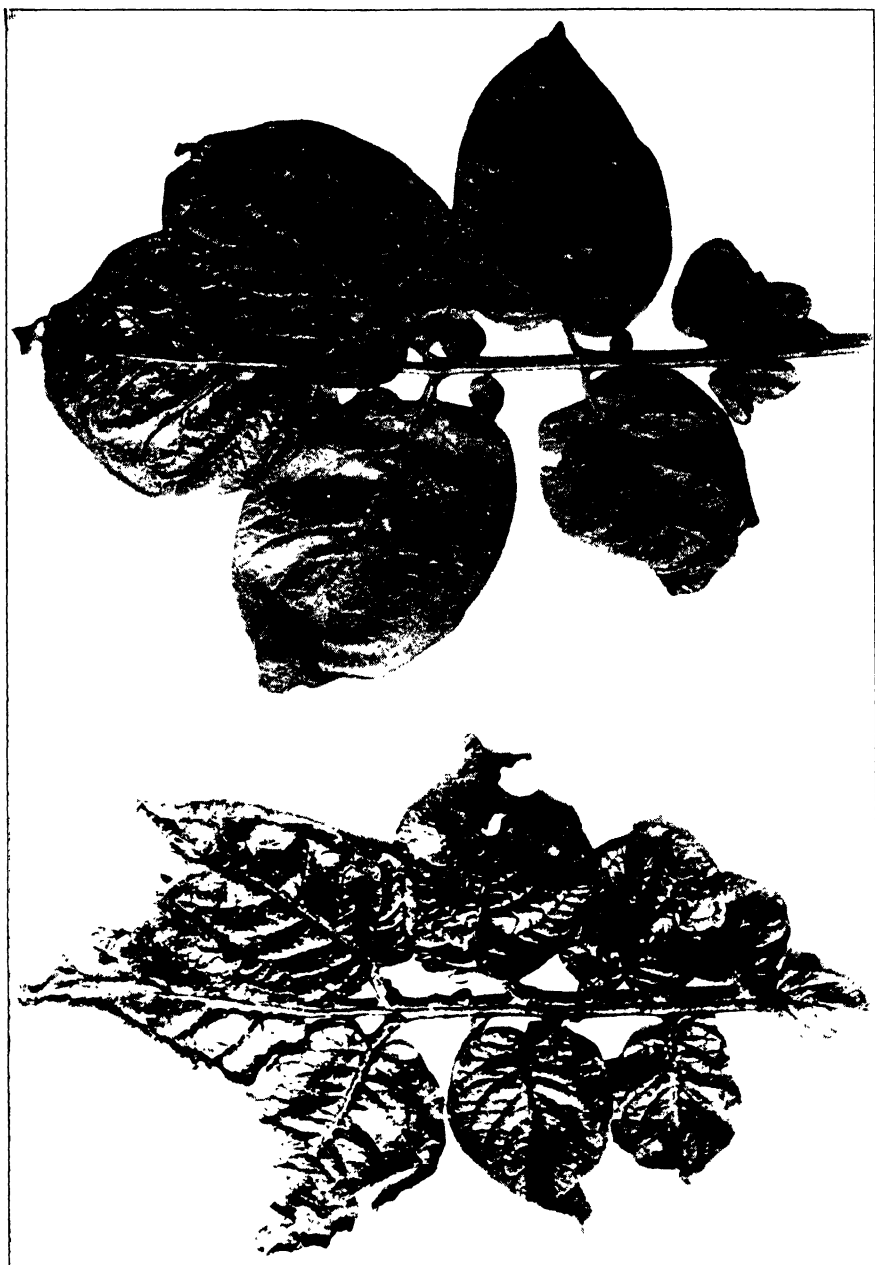
J. F. ADAMS

WITH ONE FIGURE IN THE TEXT

The frosts in Delaware during the latter part of March and the first part of April so seriously affected the apple yield, that approximately only a twelve per cent. crop for the state was harvested. However, the occurrence of one normal crop of apples during this exceptional season of 1921 has been of special interest in connection with observations on the prevalence of drouth spot.

A full crop of apples in a block of sixty-five acres of thirteen year old trees consisting of Staymans, Transparent, Ben Davis, Winesap and York Imperial was observed at the York Imperial Orchard, Seaford, Delaware. Through the kindness of Dr. W. Devitt, manager of this orchard, the following facts were secured concerning the conditions because of which this full set of fruit occurred.

The block of apple trees in this instance, was situated within one-hundred yards of a two hundred acre wood lot and extended beyond for about one-eighth mile. During the winter months the wood lot



## TIPBURN OF POTATO LEAVES

Fig. 1. Tipburn produced in cages of heavy cheese cloth (minimum amount of sunshine) by the feeding of leafhopper nymphs. Photograph taken thirteen days after the leafhopper nymphs commenced feeding.

Fig. 2. Tipburn produced in the field under natural sunlight conditions by nymphs of *E. mali*. Photograph taken 9 days after the nymphs began feeding.



had been cut over and the wood utilized for charcoal. Twenty furnaces were built on this cleared ground by placing the wood in piles measuring fourteen feet in diameter at the base and twelve feet in height. These piles of wood were then covered with about one foot of earth, thus completing the construction of the charcoal furnaces, which were burned until late in the spring. The above mentioned block of apple trees was situated southeast of the wood lot and incidentally in the same direction as the winds which prevailed during the late frost period.

Owing to this location, the blossoms on these trees were unquestionably protected from the freezing temperatures by the smudge which was produced from the burning furnaces. The remaining part of the orchard situated at one side of these protected trees was unsmudged and as a result most of the blossoms were destroyed, as likewise occurred in all other orchards throughout the state because of the existing low temperatures. However this occurrence was a coincident in so far as the resulting effects produced on the orchard, as the work on the wood lot was not conducted by the orchard company.

The failure of the trees to set fruit in the unsmudged part of the orchard indicates clearly the protection from the freezing temperatures brought about by the presence of the smudge. O'Gara (3) in connection with his work on protecting orchards from low temperatures states, "in several instances the orchards which had been smudged set a full crop, while in those that were similarly situated and not smudged the crop was entirely destroyed."

It was determined by O'Gara (3) in his work in the Pacific Northwest that the following temperatures were destructive to the apple trees: in bud 27° F., in blossom 29° F., and with setting fruit 30° F. These temperatures are approximately those of air in contact with the blossoms and fruit. The freezing temperature this spring in Delaware during the blossom period ranged from 25°-28° F. O'Gara found that a smudge was not as effective as fire to raise the temperature but can be depended upon to raise it a degree or two. A smudge is very protective in the morning before the sun comes up. It is intended only as a blanket to prevent the sun from warming up the blossoms too quickly where some slight freezing of the blossoms and the fruit has occurred during the night.

Fruit on the York Imperial trees showed a prevalence of drouth spot which disease is similar to symptoms described by various investigators as Yorkspot, Crinkle and Hollow Apple. The affected fruit showed large irregular surface depressions which when cut reveal pockets of corky tissue as shown in figure 1. The large depressions were often crescent shaped but small circular depressions were not uncommon resembling injury produced by hail stones. In all instances the de-

pressions were confined to the blush face of terminally attached fruits, but no more common to the blossom than the stem end. The normal skin over these depressions is usually a darker green. Some fruits are considerably malformed and observations during the season show a general retarded development of seriously affected fruit. Small apples that are severely affected often show over the depressions a dark brown discoloration of the skin. In such cases a gummy exudate is secreted similar to the bacterial exudation of fire-blight infection. Small droplets of the white exudation are common around the margin of the depressions

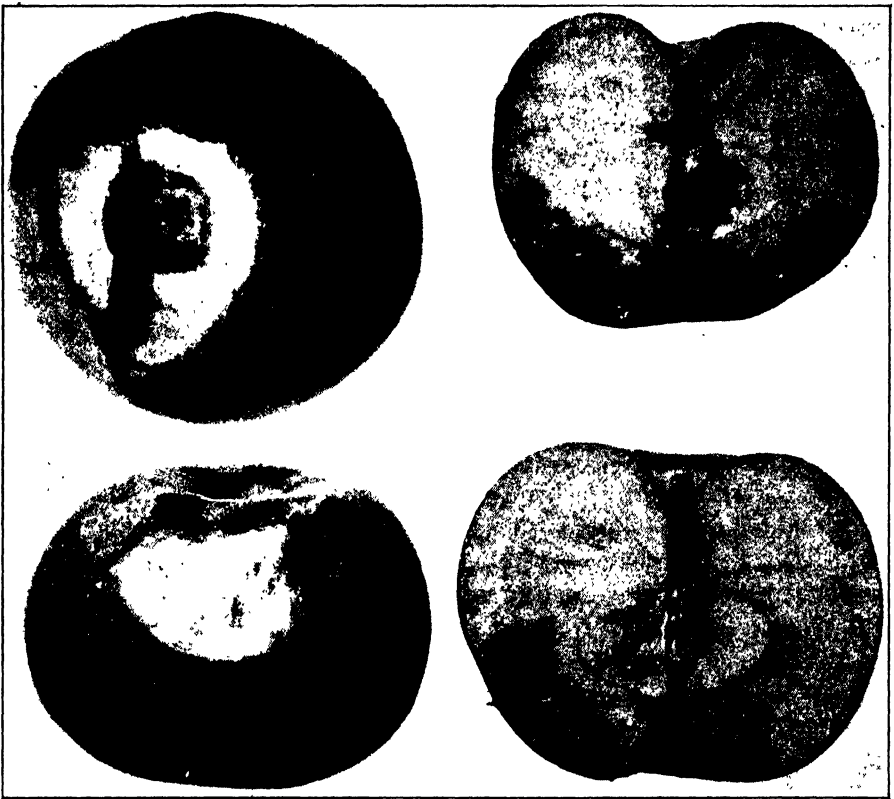


Fig. 1. APPLES AFFECTED WITH DROUTH SPOT.

but usually distributed over the surface as a thin sticky film. Such fruit hangs on but a short time and are the first to fall, usually by the middle of July under our conditions. The collapsed tissue under these depressions is light brown in appearance and often a pronounced shrinkage of the tissue occurs leaving large irregular air spaces. Affected apples that had remained undeveloped by the first part of August often showed symptoms of "glassiness" or "water core" in the pulp

adjacent to the collapsed tissue. Severely affected fruit showed evidence of such collapse that, in some instances, the black rot fungus had become established. Several diseased apples picked on the first of August were kept under temperature conditions of 65° F. After a month's time no apparent progress of the disease was observed. At the end of two months the apples showed considerable shrinkage and crinkling of the skin. The diseased areas were deeper and the skin considerably wrinkled.

The prevalence of the disease was estimated at five per cent. the first of August. A month later most of the affected fruits on the trees had fallen to such an extent, that the prevalence of the disease was reduced to one per cent. Only the large developed fruit with small depressions were found to hang on at this time.

Brooks and Fisher (1) state, "while it seems probable that Yorkspot is in part an effect of drouth, its occurrence is undoubtedly greatly influenced by sunlight and possibly by soil conditions and other agencies." Mix (2) considers drouth spot as occurring early in June and fresh stages may develop throughout the summer if the weather continues dry.

A long period of drouth occurred during the first half of the growing season. In this orchard the disease was further favored by a very light shallow surface soil and hard pan. The lack of moisture was also indicated by the short growth of crab-grass around the trees. Shallow planting, hard pan and crown gall infection are further important predisposing factors.

Considerable difference is found in the reaction of varieties to conditions of drouth. The York Imperial has been observed most seriously affected, and only a trace was found on Staymans. Mix (2) reports drouth spot in New York state on McIntosh, Northern Spy, Ben Davis, Esopus, Bellflower, Maiden Blush, Wolf River, Wealthy, Baldwin and Jonathan. Brooks and Fisher (1) report it particularly common on York Imperial apples and occurring also on the Gano and Esopus varieties.

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## SOME OBSERVATIONS ON ALFALFA GIRDLE

J. G. BROWN AND FREDERICK GIBSON

WITH PLATE XV

Among the diseases inflicting loss on the alfalfa growers of the Southwest is one whose cause is as yet unknown, although the symptoms were reported in a publication of the Arizona Experiment Station over twelve years ago. This disease is commonly referred to under the name of alfalfa girdle. It has become one of the important diseases of alfalfa in Arizona, occasionally spreading over entire fields. The past season was a very favorable one for the development of girdle in southern Arizona and the abundance of material within easy reach prompted the investigations (mainly the work of the junior author) that are briefly recorded in this preliminary paper.

Alfalfa girdle was first reported by Dr. W. B. McCallum<sup>1</sup>, at that time physiologist and pathologist in the Arizona Experiment Station, in 1909, and the disease was attributed by him to a species of *Phoma*. Dr. McCallum made no cultures or inoculations, but based his opinion on the finding of numerous pycnidia on the diseased plants. His conclusion is not supported by the few investigators who have given some attention to the disease. Osborn<sup>2</sup> in 1911 reported a girdle on alfalfa which he ascribed to the activities of an insect, *Stictocephala* sp. Other suggestions expressed orally by botanists who have had an opportunity to observe the disease in the field have included as the cause the effect of standing irrigation water, leaf hoppers, gnawing insects, etc. According to reports farmers have even attributed the trouble to smoke from smelters. The girdle has been found in most of the valleys of Arizona, in California and Colorado.

The first visible symptom of girdle formation is a slightly sunken band of light green color which widens and becomes gray-green. However, a slight enlargement can be located on the stem by using the finger tips before the shrinking of the diseased tissue occurs. As the disease progresses the girdled plants are easily located in the field, even from a distance of several rods, by the discoloration of the leaves and stem. The leaves are purplish at first, later turning yellow above, with more or less purple persisting on the under surface and at the petiolar end of the leaflet, or they may become red before turning yellow. The stem may become purpled or reddish green from the tip downward to the girdle, while the portion below the girdle remains normal in color.

<sup>1</sup> McCallum, W. B. Plant Physiology and Pathology. Ariz. Agr. Exp. Sta. Rep. 20:583. 1909.

<sup>2</sup> Osborn Herbert. Economic Importance of *Stictocephala*. Journ. Econ. Entomol. 4: 137-140. 1911.

The girdle is occasionally spiral in form, but usually it is a complete band of diseased tissue extending in a circle around the stem. The border is often slightly raised and dark colored, almost black, with gradual blending of color into normal green. Those girdles near the ground may have a thickened collar just above the shrunken band in which nematodes are sometimes found. Such thickened growths are also produced on the stem at a considerable distance from the soil line, in which case nematodes are absent. The tissue inside the dark margin varies from gray to dark brown. The epidermis always covers the shrunken part, which indicates that the injury is not due to the attack of a gnawing insect.

Another characteristic accompanying the girdle is the formation of incipient roots just above the shrunken area. This condition may exist within an inch of the tip of the stem in plants a foot or two in height. Evidently the stimulation of the tissues deeper than the epidermis and adjacent cortical cells is involved.

No girdle appeared in a certain small plot of alfalfa when it was young and tender, until about the first of August when the whole plot became so badly diseased that further cutting ceased. A few stems grew to the length of a foot or more, but they eventually became so badly diseased that they soon died. As many as eleven girdles were found on numerous single stems in the plot while one individual stem had sixteen. A large number of girdles is rare under field conditions, the average usually observed being one or two per stem, but three or four may be found. On one plant growing wild among weeds so that it made growth for a full season before being cut, four to six girdles were observed on lateral branches.

The junior author discovered girdles similar in appearance to those on alfalfa on several wild plants in the vicinity of the Experiment Station. The list of diseased specimens<sup>1</sup> now includes twelve species besides alfalfa: *Franseria tenuifolia*, *Euphorbia nutans*, *Verbesina encelioides*, *Tribulus terrestris*, *Melilotus officinalis*, *Cassia covesii*, *Bahia absinthifolia* var. *dealbata*, *Baileya multiradiata*, *Oxalis corniculata*, *Eriocarpum gracile*, *Psilostrophe cooperi* and *Melilotus indica*. Probably more plants bearing girdles of similar nature will be found, since those enumerated in this list were located within a few hours and on a comparatively small area. It remains to be seen whether all of the girdles are due to a common cause. One of the plants, *Oxalis corniculata*, showed just below a perfect girdle a scale insect (*Coccus hesperidum* Linn.<sup>2</sup>) on one

<sup>1</sup> Identified by Prof. J. J. Thorner.

<sup>2</sup> Identified by Prof. G. F. Ferris, Leland Stanford University.



side of the stem and a partial girdle on the opposite side. It is probable that the scale is connected with the disease as a carrier of the organism causing the girdle, if not otherwise related to the disease.

Histological preparations have been started but only a few are ready for examination. These show that both fungi and a bacterium are present and that the shrunk area of the girdle lies over a diseased region reaching inward to the cambium. The enlarged collar-like growth of tissue above the shrunk portion includes branches from the stele which perhaps are connected with the formation of incipient roots.

About fifty cultures have been made by sterilizing girdled portions of alfalfa stems and also those of a few other species, and placing the sterilized blocks of tissue on agar slants. From these cultures there have been isolated a species of *Alternaria*, a *Fusarium* and a bacterium whose nature has not yet been determined.

ARIZONA AGR. EXP. STA.

TUCSON, ARIZONA

JANUARY 12, 1922

#### DESCRIPTION OF PLATE XV

##### PL. GIRDLE DISEASE ON VARIOUS PLANTS

Fig. 1. *Coccus hesperidum* on stem of *Oxalis corniculata*. Note partial girdle on side of stem opposite scale and perfect girdle above.

Fig. 2. Stem of *Oxalis corniculata* showing scale illustrated in Fig. 1.

Fig. 3. Enlarged photograph of the alfalfa girdle and collar illustrated in Fig. 4.

Fig. 4. Stem of alfalfa with girdle and collar at base. The leaves were yellow at the top of the stem, purplish still farther down and green at the base.

Fig. 5. Alfalfa. a. Branching girdle; b. Ordinary type; c. Later stage than preceding girdle.

Fig. 6. a. *Oxalis corniculata*; b. *Eriocarpum gracile*; c. *Franseria tenuifolia* (*Gaertneria tenuifolia*); d. *Melilotus indica*; e. *Melilotus officinalis*; f. *Cassia covesii*; g. *Bahia absinthifolia*, var. *dealbata*; h. *Baileya multiradiata*; i. *Euphorbia nutans*; j. *Verbesina encelioides*; k. *Tribulus terrestris*.

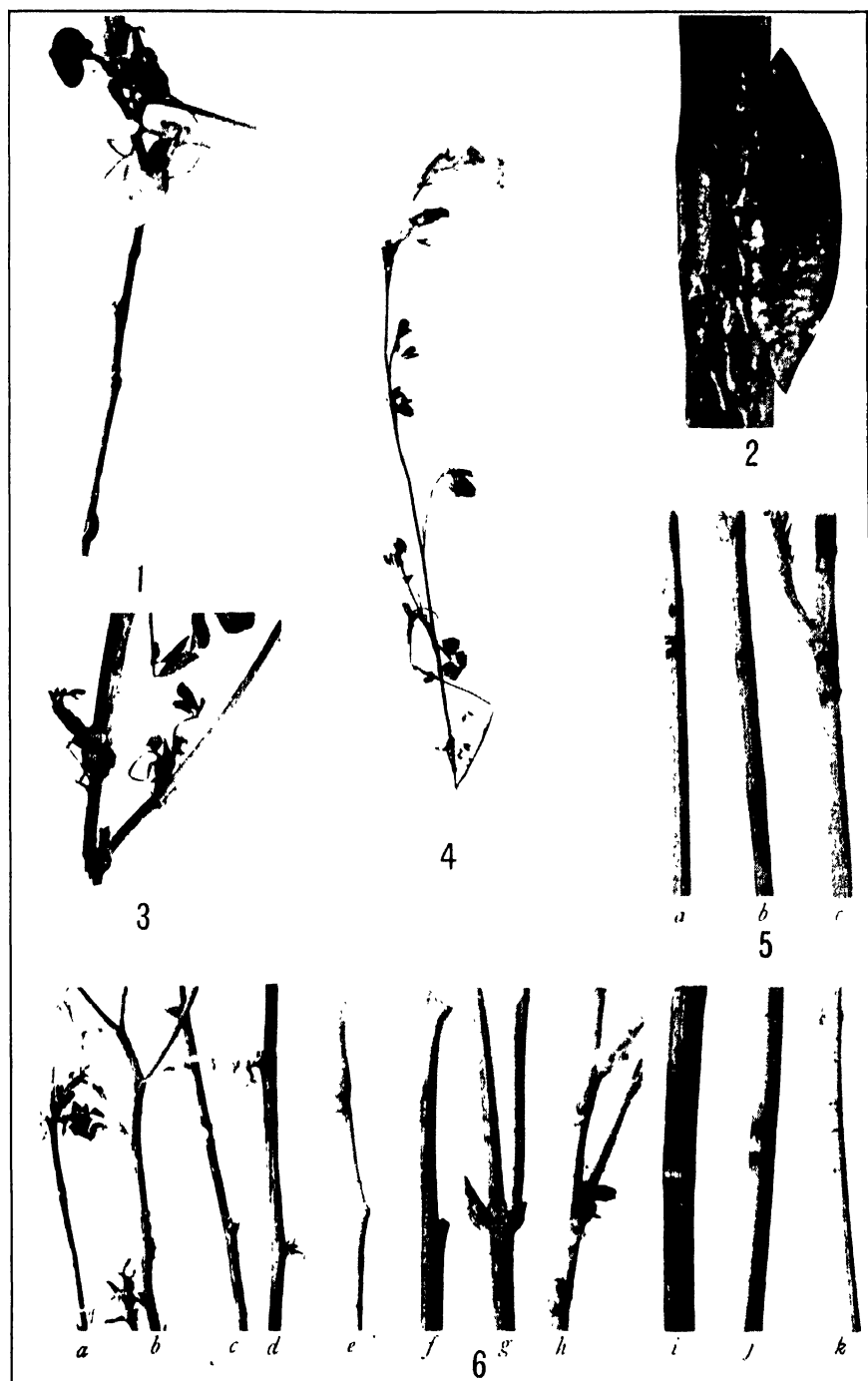
#### VARIETAL SUSCEPTIBILITY OF THE YELLOW BELL-FLOWER APPLE TO CEDAR RUST<sup>1</sup>

E. F. HOPKINS

WITH ONE FIGURE IN THE TEXT

In the spring of 1921 specimens of apple twigs apparently affected with rust were referred to the writer by Professor H. A. Cardinell of this institution with the statement that the trees were severely affected

<sup>1</sup> Published by permission of the Director of the Agricultural Experiment Station, University of Missouri.



ALFALFA GIRDLE



by the trouble. Examination of the aecidia showed that a large number of them contained spores, the measurements of which compare very well with those given for *Gymnosporangium Juniperi-virginianae* Schw. Some of the twigs were sent to Dr. L. R. Hesler of the University of Tennessee and Dr. N. J. Giddings of the University of West Virginia who confirmed the writer's identification of the material. The accompanying figure shows some of the twigs which bear lesions of cedar rust. It was noticed in several instances that lesions occurring near a bud caused it to swell and start into activity as shown on the middle twig.



FIG. 1. CEDAR RUST LESIONS ON APPLE TWIGS

The diseased specimens came from a farm in Cole County, Missouri, and occurred in a large block of apple trees consisting of a number of varieties. It was observed by Mr. T. F. Lueker, County Agent, who visited this orchard later, that only one variety, the Bellflower, was affected with rust. He says "We looked the orchard over carefully yesterday and did not find a single case on any of the other varieties." Besides some seedling trees of unknown variety there were present in this orchard the following varieties: Bellflower, Early Harvest, Delicious, Winesap, Huntsman, Green Pippin and Jeniton. This list includes

both early and late apples which were planted in among the Bellflower trees.

In connection with the above observations two points are of interest first, the abundant occurrence of cedar rust on apple twigs and second, the marked susceptibility of the Bellflower variety. According to Reed and Crabill (1) in the variety Smith's Cider which is very susceptible to rust the twigs are frequently seriously diseased. Jones and Bartholomew (2) report that young twigs may be attacked although little damage is caused on cultivated apples in Wisconsin. Weimer (3), states that in the case of susceptible varieties twigs of the current year's growth may be severely affected.

In the many reports in the literature of varietal susceptibility to apple rust the writer has found only one report of the Bellflower as a susceptible variety. This is by Jones and Bartholomew (2). In the Plant Disease Bulletin Supplement 9, 1920, p. 110, this variety is listed as being affected but its relative resistance to rust is not given. It is of interest to note that two other varieties in this orchard namely, Delicious and Early Harvest have been reported susceptible. (See Plant Disease Bulletin Supplement 9, 1920 p. 108 and 14, 1921 p. 40.)

DEPARTMENT OF BOTANY,  
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- (3) WEIMER, JAMES LEROY. Three cedar rust fungi: their life histories and the diseases they produce. New York (Cornell) Agr. Exp. Sta. Bul. 390. p. 505-549. Literature 548-549. 1917.

## PHYTOPATHOLOGICAL NOTES

*Occurrence of Phloem necrosis in leafroll tubers.* Phloem necrosis in stolons and tubers of plants affected with the leafroll disease has rarely been reported, however, observations recently made by the writer show its wide distribution even in these organs, and often a severity of symptoms more pronounced than those occurring in the lower aerial stem.

Plants of long leafroll lineage show evidence of necrosis in the stolon even before the new tubers have attained an appreciable size. The diseased groups are commonly found in the inner cycle. They show the same progressive lignification observed in the stem, and like in the latter the severity of necrosis depends on the age of the organ. Mature tubers are often borne on stolons, the phloem tissue of which is completely diseased; in the tuber itself, however, the advance of necrosis is slow, being confined primarily to the basal region, though occasionally diseased groups extend as far as to the terminal bud.

If leafroll tubers, which show only the beginning stages of necrosis, are dug up and examined again after the new plants have reached an appreciable size, it will be seen that necrosis of the phloem in the seed-piece has become greatly intensified and that the seedpieces from lateral and terminal eyes, which showed no necrosis before planting, will have it developed.

A number of experiments were conducted with whole and cut tubers to determine the relative development of necrosis in the seedpieces after the latter were planted. The tubers were cut into four parts, planted in six-inch pots, and plants and tubers examined after two months of growth. The tubers, at the time of planting, showed only slight necrosis in the basal region while the vascular tuber connection to the intercallary and terminal buds were altogether normal.

The results from these experiments only confirmed earlier observations in that necrosis developed only in vascular tuber tissue connecting to actively growing sprouts, the phloem tissue in the region of the dormant buds being normal. In case of single eye cuttings, necrosis would develop in every seedpiece, but there was a decided variation in the degree of necrosis, vigor of the individual eyes, and earliness of sprouting. There is no regularity in these variations, however, which would permit of showing distinct correlation of any of the factors; for, although the apical bud tends to be most vigorous and the basal one the weakest, the exceptions only tend to prove that vigor is a property of the individual eye, and this is depending on morphological and physiological factors, and is not necessarily affected by the position of the bud on the tuber.

Development of necrosis in the seedpiece is undoubtedly linked up with growth activities and movement of food initiated in the sprouting eye. There is an increase in vascular tissue, to accommodate the increased transfer of food substances, a condition not found in dormant buds. The relative severity of necrosis in stolons and tubers and the earliness of its development appears to be an index to the length of existence of the disease in a tuber line.—E. ARTSCHWAGER

*Personals.* Dr. Perley Spaulding, Editor-in-Chief of Phytopathology, left Washington April 4 for Europe, where he will spend the remainder of 1922, making investigations of the white pine blister rust. He will also represent the U. S. Department of Agriculture at the International Institute of Agriculture at Rome. Dr. L. L. Harter, U. S. Department of Agriculture, Washington, D. C., has been appointed by the Council as Acting Editor-in-Chief of Phytopathology during the remainder of the current year.

Dr. Arthur S. Rhoads, formerly Assistant in Forest Pathology of the U. S. Bureau of Plant Industry, and more recently of the Office of Cereal Investigations and the Office of Fruit Disease Investigations of the same bureau, has resigned to accept the position of pathologist at the Missouri State Fruit Experiment Station at Mountain Grove, Missouri.

Dr. R. D. Rands, formerly pathologist in the Office of Cereal Investigations, U. S. Department of Agriculture, and for three years under contract with the Dutch Government to conduct important investigations on the diseases of rubber and cinnamon trees in Java and Sumatra, arrived in Washington March 20.

F. J. Schneiderhan has been appointed assistant plant pathologist at the Virginia Agricultural Experiment Station for work on fruit diseases. He is stationed at Winchester in charge of the field laboratory at that place. The work includes a study of the development and control of apple scab, blister canker and other diseases.

*Pacific Coast meeting of Phytopathology.*—The Pacific Division of the American Phytopathological Society will meet in Salt Lake City, Utah, June 22–24, in conjunction with the summer session of the American Association for the Advancement of Science. It is hoped that any members of the parent society who are contemplating a trip to the Rocky Mountains or the Pacific Coast will arrange to be at Salt Lake City for this meeting. Those who wish to present papers at this meeting, please notify S. M. Zeller, Secretary-Treasurer, Pacific Division, American Phytopathological Society, Oregon Agricultural College, Corvallis, Oregon.—S. M. ZELLER.

## REPORT OF THE THIRTEENTH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The thirteenth annual meeting of the Society was held in the Mining Building of the University of Toronto, Toronto, Canada, December 27-30, 1921, in conjunction with the American Association for the Advancement of Science and affiliated scientific societies. President Donald Reddick presided and about eighty-five members were present. The headquarters of the Society were at the King Edward Hotel.

There were ninety-seven papers on the regular program, abstracts of which were printed in the January number of *Phytopathology*. Advance separates of the abstracts were mailed to all members of the Society before the meeting.

There were three joint sessions with other societies. On Wednesday afternoon, December 28, occurred the annual meeting of Section G of the American Association for the Advancement of Science. At this meeting Dr. R. H. True, retiring Vice President of Section G, gave an address entitled *The Physiological Significance of Calcium for Higher Green Plants*. There was also a symposium on *Utility of the Species Concept*, C. F. Millsbaugh speaking from the viewpoint of a systematist, G. H. Shull from the viewpoint of a geneticist, R. A. Harper from the viewpoint of a morphologist, G. B. Reed from the viewpoint of a bacteriologist and physiologist, and E. C. Stakman from the viewpoint of a pathologist. On Thursday afternoon, December twenty-nine, the American Phytopathological Society met with the Mycological Section of the Botanical Society of America for the reading of papers, half of the number being contributed by each society. On Saturday morning, December 31, a joint session was held with the American Association of Economic Entomologists for the consideration of *Insects as Disseminators of Plant Diseases*. Papers were presented by F. V. Rand on *Results of Past Investigations*, E. D. Ball on *Systematic Relations of Carriers*, L. Caesar on *Control Problems*, and M. W. Gardner on *Urgent Problems of the Future*. These four papers will appear in an early number of *Phytopathology*.

The annual dinner of the Society was held Thursday evening, December 29, in the beautiful dining hall of Wycliffe College and was attended by one hundred and ten pathologists and botanists.

The following officers were elected:

President, E. C. Stakman, University of Minnesota, St. Paul, Minnesota.

Vice President, N. J. Giddings, University of West Virginia, Morgantown, West Virginia.

Secretary-Treasurer, G. R. Lyman, United States Department of Agriculture, Washington, D. C.

Councillor for two years, I. E. Melhus, Iowa State College, Ames, Iowa.

Councillor for one year (to complete the unexpired term of Dr. Giddings), Harry B. Humphrey, United States Department of Agriculture, Washington, D. C.

Councillors for one year (chosen by the Divisions of the Society), W. H. Rankin, Dominion Laboratory of Plant Pathology, St. Catharines, Ontario, representing the Canadian Division; H. S. Reed, Citrus Experiment Station, Riverside, California, representing the Pacific Coast Division; and C. W. Edgerton, University of Louisiana, Baton Rouge, Louisiana, representing the Southern Division.

Members of the Editorial Board of *Phytopathology* (chosen by the Council of the Society): Editor for two years, L. I. Harter, United States Department of Agriculture,



Washington, D. C.; Associate Editors for three years, H. S. Fawcett, Citrus Experiment Station, Riverside, California; W. P. Fraser, University of Saskatchewan, Saskatoon, Saskatchewan; F. A. Wolf, North Carolina Agricultural College, West Raleigh, North Carolina; L. E. Melchers, Kansas Agricultural College, Manhattan, Kansas; Business Manager for one year, G. R. Lyman, United States Department of Agriculture, Washington, D. C.; Advertising Manager, R. G. Pierce, United States Department of Agriculture, Washington, D. C.

The Society voted to hold its next annual meeting at Boston, Massachusetts, December 26-30, 1922, in conjunction with the American Association for the Advancement of Science.

#### REPORT OF THE TREASURER FOR 1921

##### *Receipts:*

Balance from 1920.....	\$359.72
Membership dues.....	2,873.58
Subscriptions to Phytopathology included in checks for annual dues.....	130.00
Contributions to Phytopathology deficit included in checks for annual dues.....	39.50
Excess dues.....	14.00
Exchange.....	1.26
Interest.....	39.54
	<hr/> \$3,457.60

##### *Expenditures:*

Member subscriptions to Phytopathology.....	\$1,926.00
Secretarial work.....	89.57
Stationery, stamped envelopes, printing.....	156.66
Subscriptions included in checks for annual dues and transferred to Phytopathology account.....	130.00
Contributions to Phytopathology deficit included in checks for annual dues and transferred to Phytopathology account....	39.50
Stamps.....	5.00
Excess dues returned.....	14.00
Duplicate dues returned.....	5.00
Secretary-Treasurer's traveling expenses and miscellaneous expenses.....	49.61
Supplies.....	5.30
Checks returned by bank.....	27.06
	<hr/> 2,447.70
Balance.....	<hr/> \$1,009.90

##### *Sinking fund:*

Balance due fund from Society for 1920.....	\$165.58
Balance due fund from Society for 1921.....	535.00
	<hr/> 700.58

Actual balance.....\$309.32

On December 20, 1921, the Society had 539 members in good standing—87 life sustaining and 452 regular, as opposed to 495 members at the close of 1920, or a gain of 44 during the year. During 1921 the Society lost two members by death, Miss Eunice R. Oberly and Mr. C. E. Kurtzweil. Twenty-nine candidates were elected to membership for 1922.

## REPORT OF THE BUSINESS MANAGER OF PHYTOPATHOLOGY FOR 1921

*Receipts:*

Balance from 1920.....	\$255.96	
Member subscriptions.....	1,926.00	
Subscriptions received direct.....	1,165.23	
Sales made direct.....	303.25	
Subscriptions and sales through Williams & Wilkins Co.....	880.31	
Advertising 1920.....	193.86	
Advertising 1921, part.....	30.68	
Illustrations, excess in Hotson article.....	20.00	
Contributions to Phytopathology deficit.....	1,059.72	
Interest on invested sinking fund.....	120.00	
		<hr/> \$5,955.01

*Expenditures:*

Loan of Dec., 1920, repaid to American Phytopathological Society.....		\$800.00
Manufacturing Phytopathology:		
Vol. X, No. 10.....	\$200.92	
Vol. X, No. 11.....	421.01	
Vol. X, No. 12.....	372.46	
	<hr/>	\$994.39
Vol. XI, No. 1.....	369.60	
Vol. XI, No. 2.....	334.43	
Vol. XI, No. 3.....	366.26	
Vol. XI, No. 4.....	296.84	
Vol. XI, No. 5.....	190.09	
Vol. XI, No. 6.....	150.40	
Vol. XI, No. 7.....	372.40	
	<hr/>	2,080.02
Miscellaneous Journal Expenses.....	66.29	3,074.41
Secretarial work.....	116.23	
Supplies.....	9.85	
Excess subscriptions returned.....	3.26	
Rebate on sales of Phytopathology.....	4.50	
Printing.....	57.09	
Miscellaneous expenses of Advertising Manager.....	5.74	
Postage.....	15.00	
Checks returned by bank.....	104.00	
		<hr/> \$4,256.37
		<hr/> \$1,698.64

*Sinking fund:*

Amount invested.....	\$2,000.00
Due sinking fund from Society	
from 1920.....	\$165.58
from 1921.....	535.00
	<hr/> 700.58

An auditing committee, consisting of Mel. T. Cook and E. M. Massey, was appointed to examine the accounts of the treasurer and business manager. This committee reported the accounts correct and the reports were adopted.

## PHYTOPATHOLOGY

At the phytopathologists' dinner on Thursday evening the business manager of *Phytopathology* made the following report:

"The past year has been an important one in the history of *Phytopathology*. At the Chicago meeting the business manager reported a rapidly increasing deficit due to the steadily mounting cost of manufacturing the journal. To meet this emergency it was determined to adopt the plan which has been put into operation the past year as follows:

"1. Printing costs have been reduced. Relations with Williams & Wilkins Company of Baltimore, Maryland, our publishers for many years, were terminated and arrangements made with the Intelligencer Printing Company of Lancaster, Pennsylvania, to manufacture *Phytopathology*, beginning with the January number of 1921, at a considerable reduction in cost. Relations with the new firm have been entirely satisfactory. Unfortunately, the printers' strike forced the Intelligencer Printing Company to break in a new staff of operatives, resulting in a great delay in publication, but we are now rapidly catching up and shortly *Phytopathology* should be appearing on schedule time.

"2. Other expenses have been reduced, due to members of the Society assuming certain obligations. Formerly, Williams & Wilkins Company handled subscriptions, advertisements, and the sale of back volumes on a percentage basis. All matters relating to subscriptions are now handled by the business manager. Mr. Roy G. Pierce was appointed advertising manager and under his supervision the revenue from advertisements during 1921 exceeded the customary receipts from Williams & Wilkins Company, although the year has been one of great business depression. Through the courtesy of President A. F. Woods, the back volumes are now stored free of charge. in one of the fireproof buildings of the University of Maryland, with Professor C. E. Temple in charge. These changes have resulted in material savings.

"3. Revenues have also been increased, due to the hearty coöperation of our whole membership in securing new subscribers and new members of the Society. Fifty-five new members have been received and their names added to the mailing list of *Phytopathology* during 1921, and in addition twenty-nine others begin their membership with 1922.

"During 1920 *Phytopathology* had been obliged to borrow \$1375.00 to meet current expenses. In addition there were bills due Williams & Wilkins Company amounting to \$1060.68. Deducting \$255.96, the cash balance on hand, there remained an indebtedness of \$2179.72 on December 31, 1920. At Chicago the Society appropriated \$575.00 from its treasury, and our members, voluntarily contributed \$1059.72 toward paying this deficit, making a total of \$1634.72. There still remained an indebtedness of \$545.00 which *Phytopathology* had to carry as it entered on the new plan of operation in January, 1921.

"The saving effected under the new plan, together with the increased revenue outlined above, have enabled the journal to wipe out this deficit and the business manager takes pleasure in reporting that today *Phytopathology* is not only free of debt, but there is sufficient money on hand to pay for the remaining issues of 1921, making a volume of approximately 520 pages, with a small balance remaining in the bank. Every dollar of revenue for 1922, therefore, can be devoted to paying 1922 expenses. This means that we shall be able to continue issuing 500 to 600 pages annually, notwithstanding present high printing costs. The liquidation within one year of an indebtedness of \$2179.72 is convincing proof of the loyalty of this Society to its official journal."

## PROGRAM OF THE ANNUAL MEETING

At the annual dinner consideration was also given to the steadily increasing congestion of the program, as shown by the presence of 49 papers on the program at St. Louis, 80 at Chicago, and 97 at Toronto. This increase is natural in view of the steadily growing membership of the Society and still further increase is to be expected. It was voted, therefore, that at future annual meetings the Secretary be authorized to schedule simultaneous sessions when necessary. To relieve the congestion further and to permit more effective handling of the program, it was voted that members be requested not to submit titles and abstracts in case they have no expectation of attending the meeting. It was also voted that the printing of abstracts be continued, with any modification from the present practice which in the judgment of the Council may be necessary in the interest of economy.

## REPORT OF THE ADVISORY BOARD

The Advisory Board reported through its chairman, G. R. Lyman, on the work of the year.

The third annual summer meeting of the Society was held at St. Paul, Minnesota, and Fargo, North Dakota, July 19-22, 1921, in conjunction with the conference of cereal pathologists. Members of the Society were present from the Philippine Islands, Saskatchewan, Manitoba, and Ontario, Canada, and from ten states. There were present as guests of the Society Dr. E. J. Butler, Director of the Imperial Bureau of Mycology, London; Dr. Kingo Miyabe, Director of the Botanical Garden, Hokkaido Imperial University, Sapporo, Japan; Mr. R. J. Noble and Mr. James P. Sheldon, Department of Agriculture, New South Wales, Australia. Professor A. Jaczewski, Director of the Institute of Mycology and Phytopathology, and Professor N. I. Vavilov, Director of the Bureau of Applied Botany and Plant Breeding, both of Petrograd, Russia, arrived in America too late to attend the conference, but made an extended tour of the United States and Canada before returning to Russia.

Plans for the 1922 field meeting are developing rapidly. This meeting will be devoted to diseases of vegetables and will be held the latter part of August in the vicinity of Philadelphia.

The 1920 list of active phytopathological projects was issued during the year. The tardy responses of pathologists to requests for data indicate that the Society is not sufficiently interested in this list to warrant its continuance as an annual affair.

Dr. E. M. Freeman, chairman of the committee on the Phytopathological Institute, reported that certain prospective sources of funds have been investigated, but that the year has been an exceedingly bad one for the solicitation of money. Nevertheless we should keep this project actively before us, as its ultimate realization is by no means hopeless. At Dr. Freeman's request, he was relieved of the chairmanship of this committee and Dr. E. C. Stakman was appointed in his place.

The Advisory Board has continued to serve as the committee on phytopathology of the Division of Biology and Agriculture of the National Research Council. The most important matters considered in this connection during the year related to the Crop Protection Institute, Phytopathological Research in Tropical America, and the publication of the Farlow Bibliographical Index.

F. D. Fromme, C. R. Orton, and G. H. Coons have served as representatives of this Society on the Board of Governors of the Crop Protection Institute. This committee met with other members of the Board of Governors in Rochester, N. Y., last January and outlined a coöperative project on dusting fruits and vegetable crops for the con-

trol of diseases and insect pests. This project has been carried out in Connecticut, New York, Pennsylvania, and West Virginia under the leadership of Dr. N. J. Giddings. Some notable results were secured bearing on the control of both diseases and insects and a general summary of these results has been published in the Crop Protection Digest, vol. I, no. 2. A research project upon sulphur in its elemental and compound forms as it affects fungi and insects and as it is affected by meteorological and soil conditions, has been formulated and will be presented for approval at the next meeting of the Institute to be held at Rochester, January 12, 1922. Financial support for this project will be sought from the industries concerned, and a sufficient sum of money will be requested to establish fellowships for fundamental investigations. There is good prospect that the work will be adequately financed and that it will be initiated in the near future. Dr. Coons' term of service on the Board of Governors having expired, he was chosen by the Advisory Board to succeed himself for a term of three years.

During the past year there was organized, under the auspices of the National Research Council, The Institute for Research in Tropical America, with the object of fostering biological and other scientific investigations in the American Tropics. The American Phytopathological Society being eligible to membership in this Institute, the Council selected Dr. W. A. Orton as its representative. Dr. Orton has also served during the year as chairman of the committee of the National Research Council on Phytopathology in the Tropics, the other members of the committee being I. W. Bailey, L. R. Jones, John R. Johnston, G. R. Lyman, S. C. Prescott, Otto Reinking, and W. H. Weston, Jr. Under the auspices of the National Research Council, Dr. Orton visited Florida, Cuba, and the Bahama Islands in January and February, 1921, to investigate the need and desirability of establishing a tropical phytopathological laboratory. As a result he has recommended to the National Research Council and to the Institute for Research in Tropical America that such a laboratory be established in southern Florida. A further step toward the establishment of headquarters for tropical plant work in Florida has been taken by the Board of Control of the University of Florida, which has decided upon the establishment of a School of Tropical Agriculture at some point to be selected. Further developments in the establishment of a research center in southern Florida may be expected during the coming year.

Negotiations looking toward the publication of the Farlow Bibliographical Index are progressing under the leadership of Dr. L. R. Jones of the National Research Council, and although no definite statement can yet be made, the prospect of securing funds for publication is becoming more favorable.

#### REPORTS OF COMMITTEES

*The Committee on Standardization of Biological Stains.*—Dr. R. H. Colley, who had been temporarily appointed by the president of the Society to confer with Dr. H. J. Conn, Chairman of the Committee of the Society of American Bacteriologists on Standardization of Biological Stains, and to determine how the Society could aid that committee in its work, made a report on the present status of the biological stain situation in this country. He stated that the standardization work, formerly carried on by a committee of the Society of American Bacteriologists, had been taken over and put on a broader basis by the National Research Council, and recommended that the Society take some definite action defining its attitude toward further coöperation during the coming year. Dr. Colley was elected to represent the Society before the new committee of the National Research Council to report to this committee the results of experiments on the use of American stains, and to assist the members of the Society

with information on such results and on the sources of American stains. Inasmuch as the success of this work depends absolutely on the coöperative attitude of the members of the Society, all are requested to report the results of their experiments on American-made stains to Dr. Colley, Forest Pathology, Old Soils Building, University of Wisconsin, Madison, Wisconsin, in order that the results may be correlated and the information made available for members of the Society and for report to the central committee.

*The Committee on the F. Köplin Ravn Fellowship* reported through its chairman, Dr. L. R. Jones, that it has been in correspondence with the American Scandinavian Foundation of New York City and is assured of their sympathetic interest in this project. It has also had personal conferences with Dr. C. Ferdinandsen, Dr. Ravn's successor in Denmark, who advised that funds are being raised there for some form of memorial. It seems probable that some progress may be made toward the desired fellowship through the coöperation of these two agencies, possibly supplemented by the securing of additional funds in America. On motion the report was accepted and the committee continued.

*The Committee on Standards and Methods* made no formal report, and on motion it was voted that it be discharged from further service.

#### PROPOSED FEDERATION OF BIOLOGICAL SOCIETIES

At the request of a number of botanists and zoologists Dr. L. R. Jones, as chairman of the Division of Biology and Agriculture of the National Research Council, invited the presidents and secretaries of all biological societies meeting at Toronto to attend a conference on Tuesday, December 27, at the King Edward Hotel, to consider the advantages and feasibility of some sort of federation of biological societies. Among the advantages to be secured by such a federation were mentioned the more effective coördination of meetings and programs, and the pooling of society publications. After extended discussion the following resolutions were unanimously adopted:

*Resolved*, That it is the sense of this conference that an inter-society conference should be called to study and report upon the feasibility of federation of the biological societies and to develop plans for the said federation.

*Resolved*, That for the purpose of effecting such an organization each society and Sections F and G of the American Association for the Advancement of Science be requested to designate its president and secretary as members of an inter-society council which shall be authorized (1) to deal with all matters of common interest, such as pooling of programs, that are consistent with the existing regulations of the constituent societies; and (2) to draw up proposals for a constitution and by-laws of a federation of the societies in question and to present them for action at the next annual meetings of the societies.

The American Phytopathological Society designated Doctors Reddick and Lyman as its representatives at the proposed inter-society conference.

#### RESOLUTIONS

On motion a committee on resolutions, consisting of E. C. Stakman, J. E. Howitt, and C. R. Orton, was appointed by the president. This committee reported the following resolutions, all of which were unanimously adopted:

##### 1. *Demise of Eunice R. Oberly:*

The sudden and unexpected death of Eunice R. Oberly on November 5, has brought a feeling of personal loss to every member of the Society and sorrow to that large proportion of our membership who had the privilege of personal acquaintance with her and who have been helped by her kindness, efficiency, and wide acquaintance with

the literature of our subject. Although she was not a member of the editorial board of *Phytopathology*, Miss Oberly was a great source of help to the editors of the journal and to the Society, not only as advisor on various bibliographical technicalities, but also as the originator and early compiler of the monthly lists of "Literature on Plant Diseases," which came to be known popularly as the "Oberly list."

This expression of esteem for a colleague and friend, cut off in the midst of her achievements and plans, is inscribed with sorrow upon the Minutes of this Society.—L. R. Jones, W. A. Orton, and D. Reddick, Special Committee.

2. *Demise of Carl Kurtzweil:*

It is with deep regret and a keen sense of loss that the American Phytopathological Society takes official cognizance of the untimely death in May of one of its younger members, Carl Kurtzweil, an able and enthusiastic investigator and a splendid young man;

*Therefore, be it Resolved*, That the Secretary of the Society be instructed to send to Mr. Kurtzweil's family, expression of our sincere sympathy.—Olaf S. Aamodt, L. R. Hesler, and E. C. Stakman, Special Committee.

3. *Demise of Giuseppe Cuboni:*

The American Phytopathological Society, in annual convention assembled, expresses regret at the passing of November 3, 1920, at Rome, of the venerable plant pathologist, Dr. Giuseppe Cuboni. Active for more than forty years as an investigator, and productive throughout his life, Dr. Cuboni has long been known to the scientific world for his important contributions to mycology and vegetable pathology. The Society extends condolences to his co-workers in the Reale Stazione di Patologia Vegetale and to his colleagues in Italy.

4. *Eunice R. Oberly Memorial Prize:*

*Whereas*, A movement is under way to procure funds for a Eunice R. Oberly Cash Memorial Prize, to be granted annually for the stimulation and recognition of excellence in bibliographical work:

*Therefore, be it Resolved*, That we, the members of the American Phytopathological Society, concretely show our appreciation of Miss Oberly's work by contributing as individuals such sum as may be designated by the Council.

5. *Publications of the Department of Agriculture:*

*Whereas*, The discontinuance of the publication of the *Journal of Agricultural Research* and of the *Experiment Station Record* is a serious blow to scientific progress and international relations;

*Therefore, be it Resolved*, By the American Phytopathological Society that our retiring president be directed to transmit our views to the proper authorities.

6. *Bacterial nomenclature:*

*Whereas*, The Society of American Bacteriologists has adopted uniform rules for nomenclature of bacteria about which the American Phytopathological Society is poorly informed, but in which it is deeply interested;

*Therefore, be it Resolved*, That a special committee be appointed to study the situation and report at the next annual meeting.

7. *Distribution by cultures of plant pathogens:*

*Whereas*, There is properly constituted authority to prevent by quarantine the spread of destructive diseases; but

*Whereas*, It is impossible for any official agency to control adequately the distribution of cultures of virulent pathogens;

*Therefore, be it Resolved*, That we, the members of the American Phytopathological Society, use the utmost discretion in transportation and disposition of such cultures.

8. *Thanks to citizens of Toronto and authorities of Toronto University:*

The American Phytopathological Society wishes to express to the citizens of Toronto and to the University authorities its deep appreciation and sincere thanks for the courtesies and hospitality extended during the meetings of the Society, and especially do the members from the United States wish to thank the President of the Canadian Branch, Dr. Faull, and all the other officers and members of the Branch Society for their untiring zeal in making our meeting so pleasant and profitable. We wish to urge, furthermore, that our Canadian brethren accept this inadequate formulation of our feelings, not as a merely traditional formality, but as an honestly sincere expression of real gratitude.

9. *Thanks to the Officers of the American Phytopathological Society:*

*Whereas*, The officers of the American Phytopathological Society have sacrificed much personal peace and comfort during the last year and have earnestly and devotedly labored for the general weal;

*Therefore, be it Resolved*, By the "hoi polloi" that we express our appreciation and thanks to all the officers of the Society and that we wish them a happy, prosperous, and unpersecuted New Year.

#### MISCELLANEOUS BUSINESS

The secretary's report of the twelfth annual meeting held at Chicago, as published in Number 4, Volume 11 of *Phytopathology*, was adopted without correction.

President Reddick read a communication from Italy, inviting contributions to a memorial in Rome to Doctor Giuseppe Cuboni, late Director of the Reale Stazione di Patalogia Vegetale. On motion the president was authorized to send a contribution of \$25.00 in the name of the Society, assessing the principal phytopathological centers sufficient sums to make up the amount named.

In accordance with the resolution adopted by the Society, the president appointed as a committee on bacterial nomenclature, Dr. Erwin F. Smith, chairman, Dr. L. R. Jones, and Dr. Ernst A. Bessey.

It was voted that the president appoint a committee of two to act with the representatives of this Society on the Board of Governors of the Crop Protection Institute on the dissemination of plant diseases by seed. Dr. M. F. Barrus and Dr. M. T. Munn were appointed on this committee.

President Reddick received a cablegram from Doctors Quanjer and Westerdijk, stating that plans were being made to hold a field conference in Holland during May, 1922, and inquiring whether representatives of American phytopathologists and entomologists could be present. Upon motion the matter was referred to the Council, with authority to appoint as delegates any members of the American Phytopathological Society who could attend the Holland conference.

#### ACTION OF THE COUNCIL

The Council reported the following action for the information of members of the Society:

*Representatives on the Council of the American Association for the Advancement of Science.*—In accordance with the custom of other societies, the president and secretary of the American Phytopathological Society have acted as our representatives on the Council of the A. A. A. S., but the official duties of officers of the Societies so seriously interfered with their attendance at meetings of the Association Council that the societies were requested to designate persons other than their officers as their representatives. In accordance with this request Dr. Mel. T. Cook and Dr. C. L. Shear were named representatives of the American Phytopathological Society.



*Advisory Board.*--The Council selected Dr. J. H. Faull to succeed Dr. A. H. R. Buller as Commissioner for Canada, and Dr. R. J. Haskell to succeed Dr. G. R. Lyman as Commissioner for the United States Department of Agriculture.

*The Eunice R. Oberly Memorial Prize.*--After discussion of this matter, which was referred to it by resolution adopted by the Society, the Council directed the Secretary-Treasurer in sending out the notices of annual dues next fall to request each member of the Society to add twenty-five cents to his check as a personal contribution toward the fund for the Oberly Memorial Prize.

*Phytopathology.*--The Council recognized the importance and weight of the responsibility resting on the editor-in-chief of Phytopathology and the practical difficulties in the way of his securing any large measure of assistance from the other members of the editorial board. It was determined, therefore, to continue selecting the associate editors on the basis of regional representation as at present, but, in future to choose the other two principal editors in consultation with the editor-in-chief for terms coextensive with his own, in an effort to constitute a central editorial committee of maximum assistance to the editor-in-chief.

G. R. LYMAN, *Secretary*.

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# PHYTOPATHOLOGY

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## DECAY OF VARIOUS VEGETABLES AND FRUITS BY DIFFERENT SPECIES OF RHIZOPUS

L. L. HARTER and J. L. WEIMER

Observations have shown that *Rhizopus* is commonly associated with decayed fruits and vegetables of many kinds. Stevens and Wilcox (11) found that *Rhizopus nigricans* Ehrnb. causes a rapid deterioration of strawberries in transit, and Harter, Weimer, and Lauritzen (9) found that nine different species of the genus will decay sweet potatoes. Behrens (1, p. 515-516) showed that tomatoes were readily decayed by *R. nigricans*. The common occurrence of *Rhizopus* on fruits and vegetables has been repeatedly observed, especially on such crops as potato (*Solanum tuberosum* L.) (10), quince (*Cydonia vulgaris* Pers.) (4), pear (*Pyrus*), raspberry (*Rubus*), currants (*Ribes*), plums (*Prunus*) (1, p. 515-516), figs (*Ficus*) (2), red raspberry (*Rubus strigosus* Michx.) (11), fruits of *Cornus mascula* L., *Morus alba* L., and apple (*Pyrus malus* L.) (3). It has been observed by inspectors of the Bureau of Markets of the United States Department of Agriculture to occur on bean (*Phaseolus vulgaris* L.), beet (*Beta vulgaris* L.), cabbage (*Brassica oleracea* L.), onion (*Allium cepa* L.), squash (*Cucurbita pepo* L.), pepper (*Capsicum annuum* L.), egg plant (*Solanum melongena* L.), and other vegetables.

A review of the literature dealing with the decay of vegetables and fruits by *Rhizopus* shows that, although several crops have been mentioned as hosts, only a relatively small number of inoculation experiments have been conducted to determine its parasitism. Furthermore most of the losses due to this group of fungi have been attributed to a single species, *R. nigricans*. In view of the fact that nine species are able to decay sweet potatoes, it was decided to test the parasitism of these together with two other species nonparasitic to sweet potatoes on a number of the common vegetables and fruits. The necessity for such investigations is all the more apparent when it is recalled that

Hanzawa (5) found that the species of *Rhizopus* differ considerably in their temperature requirements. He found that they could be roughly separated into three groups with respect to temperature—high-, intermediate-, and low-temperature forms. Moreover, it has been demonstrated (9) that the low-temperature forms would not infect at high temperatures nor the high temperature forms at low temperatures, or at least only with difficulty. *R. nigricans*, one of the low-temperature forms, has practically always been cited as the species responsible for the decay of the different hosts. However, there are certain crops which are handled at relatively high temperatures, and it is probable that other species than *R. nigricans* may produce decay under these conditions.

#### METHODS OF EXPERIMENTATION

Two methods of inoculation were employed. If the crop was one which was more or less succulent or appeared to have a high water content, such as cauliflower, the inoculations were made by the insertion of spores and mycelium into a wound made with a needle. If, on the other hand, the crop was one which was relatively low in water content, such as dasheens, the spores were germinated and grown for one or two days in sweet potato decoction, which was then poured into a "well" made according to a method already described (8). The reasons for employing this method have been discussed in a previous paper and will therefore not be considered further here. In all cases enough material for an entire experiment was obtained at one time and from the same sample. In general all the products were treated alike. They were thoroughly washed and cleaned, and the experiments were carried out under as nearly aseptic conditions as possible, although in no case could absolutely sterile conditions be assured. Some of the vegetables and fruits were put into covered moist chambers and incubated at the temperature best suited for the growth of the fungus with which they were inoculated. Other crops, such as strawberries and raspberries, were placed in Erlenmeyer flasks and then inoculated by pouring a suspension of spores in water upon them.

Three temperatures were employed, 20° to 22° C; 30° C, and 35° C. These temperatures were selected because it was found that the different species of *Rhizopus* could be roughly separated into three groups with approximately these optimums.

The duration of the experiment depended upon the host, and the rapidity of decay. In most cases it continued for from three to five days. Usually it was not continued long enough to permit of complete decay of the host, but merely long enough to determine whether or not the particular crop was susceptible to decay by the different species.

As soon as the susceptibility of the host was evident, the causal organism was isolated from each individual inoculated, with the exception of such fruits as strawberries, raspberries, blackberries, etc., and identified. If the experiment was not permitted to continue too long so that the decayed tissue was overrun with saprophytic bacteria and fungi, a pure culture could usually be obtained.

#### EXPERIMENTAL DATA

The parasitism of species of *Rhizopus* has been tried on 27 different hosts, as shown by table 1. The number of individuals inoculated with each species of the genus was usually small (4 in the case of such hosts as cantaloupes, squash, turnips, etc.). The total number inoculated with all the species and the results of all the inoculations gave in most cases quite conclusive evidence as to whether or not a certain host is susceptible of decay. If in any case there was any doubt, the inoculations were repeated until reasonably conclusive proof of the susceptibility or resistance of the host to the particular species under consideration was obtained. Controls were carried for all the experiments. One set of controls was held at 30° C and another at from 20° to 22° C. If in any case the controls were decayed, the causal organism was isolated and identified. The duration of the experiments was only from three to five days, the controls usually remaining sound for that length of time. The controls which decayed were mostly of such hosts as strawberries, raspberries, and blackberries, which, although they were carefully handled when washed and prepared for the experiments, were more or less mutilated.

In all cases an attempt was made to inoculate the host by the insertion of spores and hyphae into a wound made by a needle. However, in order to obtain infection of some hosts (Irish potatoes, dasheens, beets) it was necessary to employ the "well" method.

The figures shown in the table give the percentage of hosts infected in each case and not the amount of decay at the end of the experiment. As a matter of fact, the total amount of decay at the close of the experiment varied greatly, even among species which were marked 100. Infection was considered to have taken place when the tissue about the point of inoculation was softened beyond that which resulted from artificial wounding of the controls. Care was taken not to overestimate the percentage of infection by certain organisms (*Rhizopus chinensis*, *R. microsporus*), which in comparison with other species produced a relatively small amount of decay.

The sweet potato, the host which probably suffers greater loss from decay by *Rhizopus* than any other crop, is not included, since an extensive study was previously made of its susceptibility to decay by the different species of *Rhizopus*, the results of which have been published elsewhere (9, 7).

## DISCUSSION OF RESULTS

The results recorded in table 1 show that a number of the fruits and vegetables are susceptible to decay by the different species of *Rhizopus*.

TABLE 1.

*Percentage of infection by species of Rhizopus on various hosts*

Hosts	Incubation temperatures										
	35°	30°							20°-22°		
	Chinensis	oryzae	maydis	tritici	delemar	nodosus	arrhizus	artocarpi	reflexus	microsporus	nigricans
Grapefruit.....	100	100	100	100	100	100	...	100	100	00	100
Orange.....	100	100	100	100	100	100	...	100	100	75	100
Lemon.....	50	100	100	100	50	100	...	75	100	100	100
Turnip.....	75	100	100	100	100	100	...	75	100	00	25
Parsnip.....	100	100	100	100	100	100	...	100	100	00	100
Rutabaga.....	100	100	100	100	100	100	...	100	50	00	75
Carrot.....	50	100	100	100	100	100	100	50	100	00	25
Pepper (green).....	100	100	100	100	100	100	100	100	100	100	100
Cucumber.....	50	100	100	100	100	100	100	100	100	00	75
Potato.....	40	100	90	100	100	100	90	90	90	00	65
Apple.....	00	100	100	100	100	100	100	100	100	00	100
Dasheen.....	50	100	100	100	100	100	75	100	75	00	100
Peas.....	00	100	100	100	100	100	100	00	00	00	00
Bean.....	100	100	100	100	100	100	100	50	50	00	100
Beets.....	00	75	25	25	50	50	50	00	00	00	00
Yam (Dioscorea).....	...	...	...	90	...	...	...	...	...	...	90
Strawberries*											
Squash.....	00	100	100	100	100	100	100	100	100	50	100
Blackberry*											
Raspberry (red)*											
Cauliflower.....	00	...	...	100	...	...	...	...	...	00	100
Peach.....	100	100	100	100	100	100	100	100	100	00	100
Cantaloupe.....	100	100	100	100	100	100	100	50	100	50	100
Watermelon.....	00	100	00	100	100	100	50	100	100	00	100
Eggplant.....	00	100	100	100	100	00	100	100	00	00	50
Plum.....	...	...	...	...	...	...	...	...	...	...	...
Pear.....	100	100	100	100	100	100	100	100	100	50	100

\* Decay readily. Impossible to obtain reliable results with different species.

Some hosts are decayed with more difficulty than others, for instance beets. On the other hand, there are some which succumb readily to all the species. At the outset of these experiments the writers were not in possession of a culture of *Rhizopus arrhizus*, therefore no results were obtained for a few of the hosts with this species. The species

*microsporus* and *chinesnis* are for the most part nonparasitic, and it is doubtful whether either of them will be very often found on decaying fruits and vegetables on the markets. However, under the conditions of these experiments, in which they were removed from the competition of other fungi, they produced decay of certain hosts.

The different species have been arranged in three groups, according to what seemed to be the optimum temperature for infection—i. e., high-, intermediate-, and low-temperature forms. *Rhizopus chinensis* is the only representative of the high-temperature group. The optimum temperature for the growth of this species is distinctly higher than that of any of the other species. In the intermediate groups are *oryzae*, *maydis*, *tritici*, *delemar*, *nodosus*, and *arrhizus*, whose optimum temperature was found to be about 30° C. The low-temperature group is represented by *artocarpi*, *reflexus*, *microsporus*, and *nigricans*, whose optimum is about 20°–22° C. *R. chinensis* is weakly parasitic on a few hosts. If, however, an examination is made of the results obtained with the intermediate-temperature forms it will be seen that they are all vigorous parasites. As a matter of fact, these species produced a higher percentage of infection and a more rapid decay than the species of the other groups. However, so far as the writer's experience goes, none of the species in the intermediate group are met with as frequently in nature as *nigricans*, which seems to be the immediate cause of most of the decay produced by this group of fungi. It is exceedingly surprising that species which under laboratory conditions are such vigorous parasites are so seldom found on decayed fruits and vegetables in storage or on the markets. *Nigricans*, the species most often isolated, produced a smaller percentage of infection than either *artocarpi* or *reflexus*, two species only occasionally isolated from decayed material.

The hosts differed considerably in the ease with which infection took place and the method necessary to accomplish it. Some hosts, such as beets and potatoes, could be infected only after the spores had been germinated in a nutrient solution and had grown on the medium for from 24 to 48 hours, while other hosts (cantaloupes, cucumbers, oranges) can readily be infected by merely inserting spores and hyphae into a wound made by a needle. The method of inoculation required seems to depend upon whether or not the host is juicy or relatively dry. On Irish potatoes and beets it seems that it is necessary for the enzyme pectinase to be secreted in sufficient quantity to macerate the tissue in advance of the growing hyphae, as the writers have shown to occur in infected sweet potatoes (6). However, on hosts with a higher water content, the organism seems to be able to infect when the spores and mycelium are inserted directly into the tissue. The different species

vary in their ability to infect certain hosts by merely inserting spores and hyphae into wounds. For example, *tritici* infected apples readily when spores and hyphae were inserted into them, while *nigricans* did not, although the latter species did infect when the "well" method was employed. The dasheen was likewise more readily infected by the prick method with *tritici* than with *nigricans*.

The data obtained from the inoculations of strawberries, raspberries, and blackberries are not entirely satisfactory. There is no reason to doubt but that these three hosts are decayed readily by *Rhizopus*, but to prove that each of the species of *Rhizopus* will decay them has been surrounded by many difficulties. In the first place, the controls decayed readily, showing the presence of *Rhizopus*. In some few cases the writers isolated the species with which the inoculations were made in pure culture. More often, however, either *nigricans* or *tritici* or a mixture of two or more species was isolated. It was possible in some cases to recover the species with which the inoculation was made mixed with either *nigricans* or *tritici* or with both. The results with these three hosts proved their susceptibility to decay, but they can hardly be interpreted as showing the parasitism of all the species.

*Rhizopus* seems to be characterized by its inability to infect except through wounds. Of all the different hosts tried, only one, the peach, seemed to be infected without any apparent wound. It is of course difficult to say with certainty that there was no wound through which the germ tube entered, but the evidence seems to indicate that infection can take place through the unwounded surface. What appeared to be healthy, ripe peaches were dipped in a spore suspension of *nigricans* and of *tritici* and infection resulted. *Rhizopus* seems to be further characterized by its inability to grow outward through the unbroken skin. On the other hand, if the skin or epidermis of an infected host is broken, the hyphae grow through the wound and sporangiophores are formed abundantly on the surface. Here again peaches constitute an exception. It was found that the sporangiophores grew outward through the skin at all points on the surface and fruited abundantly.

The writers' results with peaches and to some extent with plums indicated that the decay generally regarded as brownrot may be caused in part at least by *Rhizopus*. Isolations were made from 139 partially decayed peaches collected on the retail markets of Washington. Two plates remained sterile, while *Sclerotinia cinerea* (Bon.) Schroet. was obtained from 76, *Rhizopus* from 44, bacteria from 5, and *S. cinerea* and *Rhizopus* from 12, showing that the two organisms may be jointly responsible for the rotting of peaches. *Nigricans* was the predominating species. The species obtained from one or two of the isolations belonged to the *tritici* group and three resembled *artocarpi* but differed from the

latter species in some important details. The appearance of the decay caused by *S. cinerea* and *Rhizopus* is so much alike that it is readily understood how one might suspect one organism and entirely overlook the other. In the early stages of decay there are no outstanding differences which would enable one to separate the two. When the decay becomes more advanced, the skin of peaches decayed by the brown rot fungus turns slightly darker, while that of peaches decayed by *Rhizopus* remains brown. In the last stage of decay the fruiting on the surface is so different and characteristic as to be unmistakable. Under the conditions of the writers' experiments with the two organisms *Rhizopus* produced the more rapid decay.

Isolations were made from 66 plums collected from the retail markets of Washington and *Rhizopus* was obtained from 27, *Sclerotinia cinerea* from 34, and 5 plates remained sterile.

These results show that *Rhizopus* is prevalent on decayed peaches and plums on the markets. It has also been frequently isolated from other fruits and vegetables. Squashes are especially subject to decay by *Rhizopus*, while watermelons, cucumbers, cantaloupes, etc., have been often collected in the retail markets more or less completely decayed by this fungus. *R. nigricans* is the predominant species isolated, though species of the *tritici* group are occasionally met with. The results of these experiments, together with the fact that *Rhizopus* has been observed so frequently on and isolated from decayed material in storage or on the markets, show that this organism is one of the most destructive and prevalent of the rot-producing fungi.

#### SUMMARY

(1). A study was made of 11 species of *Rhizopus* on 27 different hosts. All the hosts were susceptible to decay by some of the species. Two species, *microsporus* and *chinensis*, were more or less nonparasitic—i. e., they infected only a few of the hosts and in those cases somewhat weakly.

(2). The species of the intermediate-temperature group are, on the whole, more vigorous parasites under artificial conditions than the members of the low-temperature group. Observations and investigations thus far seem to indicate that *nigricans* is the predominating species causing decay of fruits and vegetables in storage and on the markets. This species was obtained in most cases from decaying material collected from the markets. Occasionally one of the species of the intermediate-temperature group was isolated.



(3). The method of inoculation necessary to produce infection depended upon the host; beets, Irish potatoes, and those crops apparently low in water content could be infected only by the "well" method. Others, on the other hand, that were watery or juicy could be infected by merely inserting the spores and hyphae into a wound made by a needle.

(4). None of the hosts could be infected without wounding, with the possible exception of ripe peaches. Peaches with no apparent wounds when dipped into a spore suspension became infected, and sporangio-phores were formed on the surface. A considerable percentage of the peaches and plums on the retail markets decayed with what appeared to be the brownrot organism were found to have been decayed by *Rhizopus*.

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# ROOT ROT OF PINE SEEDLINGS

ANNIE E. RATHBUN

WITH ONE FIGURE IN THE TEXT

For several years coniferous seedlings too old to succumb to the more ordinary types of damping-off—namely germination loss, normal damping-off and top damping—recognized by Hartley (4) have been known to suffer from late damping-off and root sickness or rot. Büttner (1) studied the root rot of coniferous seedlings and while he apparently made no isolations or inoculations, held *Fusoma parasiticum* responsible for at least part of the rot. Gifford (2) isolated a species of *Fusarium* from the roots of 2 year old seedlings of *Pinus strobus* L. and 1 year old seedlings of *P. sylvestris* L. Although he apparently performed no inoculation experiments he not only thought that this species of *Fusarium* was capable of causing damping-off and root rot but also expressed the opinion that it may sometimes even kill the older seedlings by attacking the roots and stems. Hartley (3) stated that a “*Rhizoctonia* species (probably *Corticium vagum* B. and C.) which causes damping-off of very young seedlings, sometimes continues to work in patches till the seedlings are 2 months old or even more.” Therefore in order to establish experimentally what these field observations seemed to indicate, namely that damping-off fungi may be responsible for these root injuries, 4 experiments were carried on with pine seedlings. The results of an earlier experiment of this sort have been reported by Hartley (4). Most of the fungous strains used in the experiments described below have been tested as damping-off parasites by Hartley and his associates, (4), (5). Results with the same strains in damping-off inoculations by other methods will later be published by the writer.

The seedlings for all 4 experiments were grown in the greenhouse in autoclaved sand until the time of inoculation. They, however, had been watered for several months with unboiled water. At the time of inoculation, the seedlings were carefully dug up and the root systems washed with boiled water. In the first 3 experiments (numbers 112, 113, 114) a little autoclaved sand was placed in the bottom of 4 inch pots and on top of it in each pot were placed 10 apparently healthy seedlings. Next inoculum which for each pot consisted of fragments of the mush made from one-third teaspoonful of rice plus the mycelium and any spores growing upon it, was laid against a portion of the root

systems of the seedlings. The inoculum was taken from cultures approximately 1 week old, but the strains themselves had been isolated several years. An equal amount of sterile rice mush cut into fragments was added to each control pot. Inoculum and roots were then covered with autoclaved sand. Unboiled water was still used to water the seedlings. In experiment number 112 two units of 10 six months old seedlings of *Pinus resinosa* Ait. were inoculated with each fungus. Experiment number 113 was a duplicate of number 112 except that about 2.5 inches were cut from the root system of each seedling before inoculation, in order to ascertain whether wounding would increase the susceptibility of the seedlings, and that but one unit of 10 seedlings was inoculated with each fungus. In experiment number 114 four-months old *Pinus banksiana* Lamb. seedlings were substituted for those of *P. resinosa*. The data were taken at the end of 40 days, the roots of the living seedlings being carefully washed out to permit examination. In experiment number 119, 10 seedlings of *P. resinosa* were placed in Erlenmeyer flasks in which fungi were growing on rice mush. The whole root system was in contact with the inoculum. The counts were made at the end of 10 days and are hence less reliable than those for the other 3 experiments because they do not show the full effect of the fungi.

The fungi tested were *Pythium debaryanum* Hesse, *Corticium vagum* B. and C., *Fusarium* spp., *Botrytis* spp., *Phomopsis juniperovora* Hahn and *Rheosporangium aphanidermatus* Edson.

#### RESULTS

The results of these experiments are given in figure 1 and table 1. Unfortunately they are based upon the inoculation of too few seedlings to admit discussion of the comparative virulence of the different strains, but they do permit to a certain extent at least a comparison of the different genera. A small amount of root rot developed in the controls and a similar amount in some of the inoculated pots. However *Corticium vagum* caused a decided increase in the amount of root rot while *Pythium debaryanum* and the cinerea type of *Botrytis*, caused more or less decay to most of the roots. Strains of *Fusarium* spp. gave variable results, but as a whole in the pots inoculated with *Fusaria* root rot was more serious than in the average control. A more detailed discussion of the results with the various genera based upon a direct comparison of the number of seedlings with undecayed roots remaining after inoculation (Fig. 1) is given below.

*Pythium debaryanum*. In experiment number 112, each *P. debaryanum* strain caused more decay than was present in the two controls with the least number of undecayed roots. In the average control for number 112, there remained at the end of the experiment five times as many

seedlings with undecayed roots as in the average *P. debaryanum* pot; in that of number 113 there were about twice as many; in that of number 114, there were about eight times as many, while in that of number 119 there were about five times as many. Several of the *P. debaryanum* strains caused browning of the tops as well as decay of the root systems.

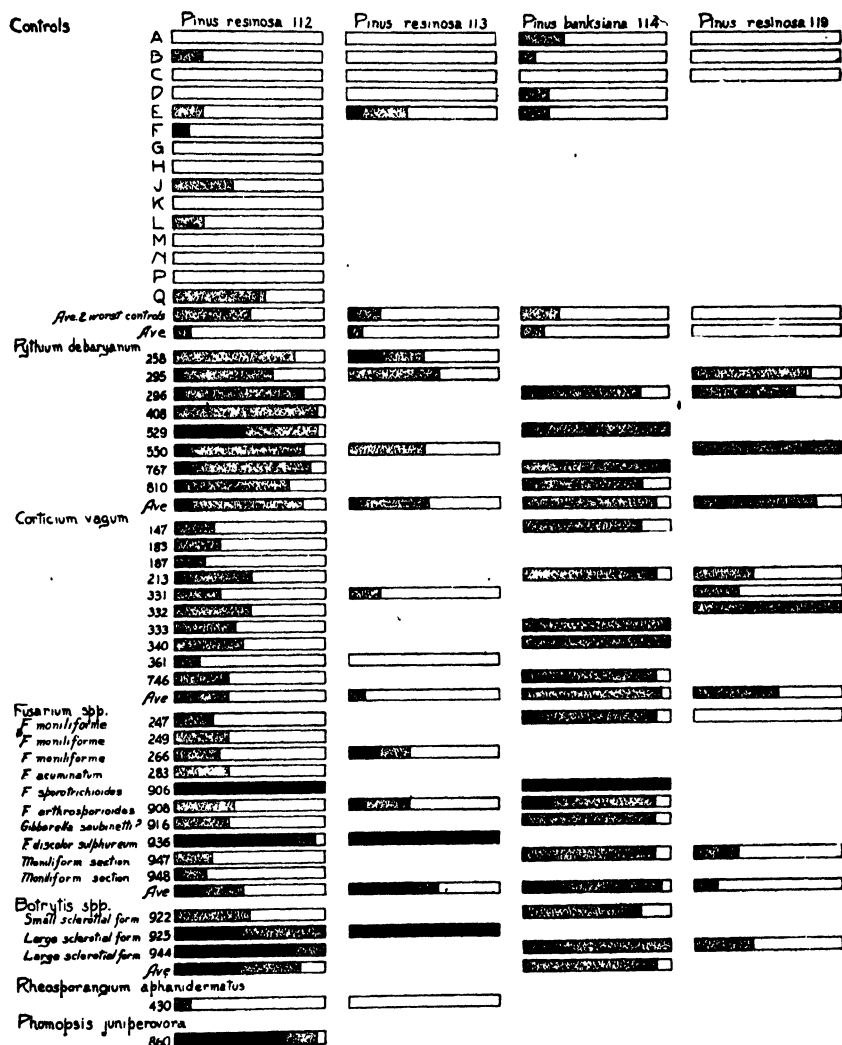


FIG. 1. Diagram showing effect of fungi on the root systems of conifers. Black = number of seedlings with root system wholly decayed. Stippling = number of seedlings with root system partially decayed. White = number of seedlings with roots undecayed or only doubtfully decayed. Each rectangle represents one unit of 10 seedlings in experiments numbers 113, 114, 119, and the average of two units of 10 seedlings each in experiment number 112.

This latter is the late damping-off described and illustrated by Hartley (5). These experiments not only confirm Hartley's (4) report that *P. debaryanum* can cause root rot of one and a half months old *Pinus resinosa* seedlings, but show that it can attack 6 months old ones as well (Tab. 2).

*Corticium vagum*. In experiment number 112, the number of seedlings with undecayed roots in the average control was slightly greater than in the average *Corticium vagum* pot; in number 113 the numbers were approximately equal; in number 114, there were more than 10 times as many seedlings with undecayed roots in the average control; while in number 119, there were about twice as many. No late damping-off was observed. Although strains number 147 and 213, caused less rot

TABLE I  
Summarized Results of the Experiments

Inoculating fungus	Experiment number	Number of seedlings inoculated	Percentage with wholly decayed root system	Percentage with partially decayed roots	Percentage healthy or only doubtfully decayed
Controls	112	150	0	11	89
	113	50	2	6	92
	114	50	0	16	84
	119	30	0	0	100
<i>Pythium debaryanum</i>	112	160	10	74	16
	113	30	7	46	47
	114	40	0	92	8
	119	30	0	83	17
<i>Corticium vagum</i>	112	200	1	34	65
	113	20	0	10	90
	114	50	0	92	8
	119	30	0	57	43
<i>Fusarium</i> spp. F. moniliform species and section	112	100	1	26	73
	113	10	20	20	60
	114	20	5	85	10
	119	20	0	15	85
<i>F. acuminatum</i>	112	20	0	35	65
<i>F. sporotrichioides</i>	112	20	100	0	0
	114	10	100	0	0

TABLE I (Continued)

Summarized Results of the Experiments

Inoculating fungus	Experiment number	Number of seedlings inoculated	Percentage with wholly decayed root system	Percentage with partially decayed roots	Percentage healthy or only doubtfully decayed
<i>F. arthrosporioides</i>	112	20	0	40	60
	113	10	10	30	60
	114	10	70	20	10
<i>F. discolor sulphureum</i>	112	20	80	15	5
	113	10	100	0	0
Conidial stage of <i>Gibberella saubinetii</i> ?	112	20	0	35	65
	114	10	0	90	10
Average <i>Fusarium</i> species	112	200	19	26	55
	113	30	43	17	40
	114	50	26	66	8
	119	20	0	15	85
Botrytis (Large sclerotial form)	112	40	63	37	0
	113	10	100	0	0
	114	10	0	100	0
Botrytis (Small sclerotial form)	112	20	0	50	50
	114	10	0	80	20
<i>Rheosporangium aphanidermatus</i>	112	20	0	10	90
	113	10	0	0	100
<i>Phomopsis juniperovora</i>	112	20	75	20	5

than they did in Hartley's (4) experiment, they still caused enough to confirm his statement that *Corticium vagum* is able to cause root rot (Tab. 2).

*Fusarium* spp. In experiment number 112, the number of seedlings with undecayed roots in the average control was greater than in the average *Fusarium* pot; in number 113 twice as great; in number 114, ten times as great; and in number 119 slightly greater. In every case

the moniliform section contained fewer seedlings with undecayed roots than the average control, but more than the average *Fusarium* pot. Strain number 249, seemed to cause more injury than it did in Hartley's (4) late damping-off experiment (Tab. 2). *Fusarium acuminatum* E. and E., *F. arthrosporioides* Sherb. and *Gibberella saubinetii* ? (Dur. & Mont.) Sacc. seemed slightly parasitic, while *F. discolor sulphureum* (Schlecht.) Ap. & Wr. and *F. sporotrichioides* Sherb. were decidedly parasitic. Both of the latter caused late damping-off as well as root rot. Gifford (2) as mentioned above has previously found a species of *Fusarium* causing root sickness of 2 year old white pines.

TABLE 2

Comparison of results obtained by these methods with those formerly obtained by Hartley (4) on *Pinus resinosa*

	Hartley's results*		Writer's results	
	No. seedlings inoculated	Per cent healthy or only doubtfully injured	No. seedlings inoculated	Per cent healthy or only doubtfully injured
<i>Pythium debaryanum</i>				
295	57	65	30	37
550	46	72	30	27
810	37	59	20	25
Total	140	66	80	30
<i>Corticium vagum</i>				
147	53	49	20	75
213	44	45	20	50
Total	97	47	40	63
<i>Fusarium moniliforme</i>				
249	32	88	20	65
Controls	115	88	200	89

\* Compiled from Hartley's (4) table 10 and the original data from which his table 10 was constructed.

*Botrytis* spp. The small sclerotial form of *Botrytis* caused little if any injury to the roots of coniferous seedlings, while the cinerea types (or large sclerotial forms) were decidedly parasitic, injuring practically all of the seedlings. One strain of the latter was able to cause late damping-off.

*Rheosporangium aphanidermatus* caused no appreciable decay, while *Phomopsis juniperovora* caused considerable decay in one experiment. This together with the results secured with *F. discolor sulphureum* and

*F. sporotrichioides* suggests the possibility that under conditions favorable to the parasite other fungi as well as damping-off parasites may cause root rot of coniferous seedlings. However, the fact that the damping-off parasites are known to be present in the soil makes them the most probable enemies.

Confirmation of the above statements was secured in general by determining the total number inoculated with each genus and from that recalculating the percentage with undecayed roots and the percentage injured. Table 1 is based upon such recalculated percentages.

Cutting off a portion of the root system did not increase the susceptibility of *Pinus resinosa*. There seemed, on the other hand, to be a slightly decreased susceptibility. This is probably explained by the fact that the fungi usually enter the roots through the young succulent roots which in this experiment were missing. *Pinus resinosa* and *P. banksiana* seemed approximately equally susceptible to *Pythium debaryanum*, but *P. banksiana* seemed on the whole more susceptible to *Corticium vagum* and the *Fusarium* spp. It is impossible to say definitely whether or not these variations were accidental because the number of seedlings was too small to establish relative susceptibility.

These experiments should be repeated under less artificial conditions not only for the purpose of establishing the relative susceptibility of the two hosts, but also for the purpose of definitely establishing the fungi in question as the cause of root rot.<sup>1</sup>

#### SUMMARY

Roots of *Pinus resinosa* and *P. banksiana* too old to damp-off were inoculated with fungi, mostly damping-off parasites. Inoculum on rice mush was applied directly to the roots, which in most of the experiments were again covered with sterilized sand. A small amount of root rot developed in the controls and a similar amount on a few seedlings inoculated with *Rheosporangium aphanidermatus* and a small sclerotial *Botrytis*. *Corticium vagum* caused a distinct increase in the amount of

<sup>1</sup> In the above experiments the writer used Carl Hartley's cultures. He obtained them from the following people: Numbers 906, 908, 916 from D. Atanasoff; 183 from M. F. Barrus; 295, 296, 213, 936 from H. A. Edson; 810 from L. A. Hawkins; 947, 948 from G. N. Hoffer; 944 from W. T. Horne; 187 from Mrs. C. R. Tillotson; 922, 925 from H. H. Whetzel. The remainder of the cultures were isolated by various members of the Office of Forest Pathology. The writer also wishes to thank Dr. Hartley for suggestions and criticism of the work; Rush P. Marshall for making the drawing for figure 1; Miss Helen Johann for identifying Mr. Atanasoff's cultures and C. D. Sherbakoff, who identified cultures numbers 947 and 948 as of the moniliform section, but apparently different organisms.



rot, while *Pythium debaryanum* and the cinerea type of *Botrytis* caused more or less decay to most of the roots. Strains of *Fusarium* spp. gave variable results, but as a whole in the *Fusarium* pots root rot was more serious than in the average controls. One strain each of *F. sporotrichioides* and *F. discolor sulphureum* killed nearly all the seedlings. Very serious rot also occurred in a small unit of 20 seedlings inoculated with *Phomopsis juniperovora*.

While it is not to be expected that the results of these very artificial tests are representative of what goes on in ordinary nursery beds, they are taken to indicate that the root sickness commonly found in pine seedlings is at least in part caused by damping-off fungi.

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## VIABILITY OF TELIA OF CRONARTIUM RIBICOLA IN EARLY WINTER

PERLEY SPAULDING

An attempt has been made to determine how late in the season the telia of *Cronartium ribicola* may remain viable under natural outdoor conditions. The work was carried on at Bethel, Vermont, a fairly representative locality for northern New England and New York, where winter begins early in the season. The investigations began the 26th of September and continued until the 8th of December, 1921. The latter part of the summer and major portion of the fall in the northeastern states had been abnormally dry. Snow fell on November 7 and remained on the ground until November 19. Snow again fell within a few days and was still on the ground when the studies were discontinued, so that winter weather prevailed for a full month before the final tests were made.

The experiments were carried out as follows: Vigorous living *Ribes* leaves bearing an abundant supply of telia were secured in different localities, as follows: *Ribes nigrum*, *R. odoratum* and *R. americanum* from Block Island, Rhode Island, *R. rotundifolium* from North Hudson, New York, and *R. cynosbati* from Bethel, Vermont. The telia were germinated by placing the leaves upside down in Petri dishes with wet absorbent cotton and filter paper in the bottom beneath the leaves and wet filter paper in the cover above the leaves. The dishes were kept in an unheated room except for the last three tests (Tab. 1), when it was decidedly below freezing out of doors. In these cases the dishes were kept in a moderately warm room. The leaves were enclosed in mosquito netting bags and hung out of doors. Tests were made on September 26, October 3, 21, and 29, November 8, 21, and 30, and December 8. The results shown in table 1 are: telia on *Ribes cynosbati*, *R. rotundifolium*, and *R. odoratum* nearly reached their limit of viability, the latter being the only one which showed some germination on December 8. Those on *R. americanum* retained considerable vigor on this date and apparently were good for a considerable period longer. The outstanding feature of the results, however, were those obtained with *R. nigrum*. Telia collected September 21 still germinated vigorously on December 8. Other telia collected October 11 were almost as vigorous on December 8 as when they were first collected. (Tab. 1.)

Some miscellaneous observations and tests of freshly collected telia, as shown in table 2, help to completely show the significance of these results. In the dry weather of September and October, it was noted repeatedly that telia located upon dead leaves or dead spots of living leaves gave no germination. (Tab. 2.) This was true not only of species like *R. cynosbati* and *R. rotundifolium*, but was also true of *R. nigrum*. At the same time vigorous living leaves on the same bushes bore telia which were strongly viable. It was also found that fresh green leaves

TABLE 1

*Longevity of telia of Cronartium ribicola kept out of doors under natural conditions*

	Telia from						
	<i>R. nigrum</i>	<i>R. cynosbati</i>	<i>R. rotundifolium</i>	<i>R. nigrum</i>	<i>R. odoratum</i>	<i>R. americanum</i>	<i>R. rotundifolium</i>
Date collected	S 21	S 25	S 29	O 11	O 11	O 11	O 11
Results of Tests							
Tested Sept. 26	xxx*	x					
Tested Oct. 3	xx	No test	x				
Tested Oct. 21	xxx	x	x				
Tested Oct. 29	xxx	x	x	xxx	x	xx	xx
Tested Nov. 8	xxx	xx	0	xxx	x	xx	x
Tested Nov. 21	xx	x	0	xxx	x	xx	xx
Tested Nov. 30	xx	0	0	xxx	x	x	0
Tested Dec. 3	xx	0	No test	xxx	x	x	0

\*xxx Maximum germination

xx Medium germination

x Light germination

(x) Very slight germination

0 No germination

bearing telia, plucked from the bushes and allowed to air-dry naturally, bore telia which germinated strongly. This suggested the conclusion that telia upon leaves that were killed suddenly would retain all of their original viability and that leaves thus killed suddenly by frosts would be found to bear telia of maximum germinating powers. When

frosts finally came, this proved to be the case. It is well known that subjection to cold temperatures approximating freezing stimulates germination of all the different forms of spores of *Cronartium ribicola*. Disregarding *R. nigrum* from consideration, telia on *R. cynosbati* were no stronger in germinating power than those on any other species of

TABLE 2

*Vigor of telia of Cronartium ribicola under varying conditions at the time of collection.*  
*Test made immediately after collection*

Specimen	Collected		Tested	
<i>R. nigrum</i> , vigorous leaves.	Block Is., R. I.	Sept. 21	Sept. 26 Oct. 1	xxx xxx
<i>R. cynosbati</i> , vigorous leaves.	Bethel, Vt.	Sept. 25	Sept. 26	xx
<i>R. cynosbati</i> , dying leaves.	Bethel, Vt.	Sept. 25	Sept. 26	0
<i>R. cynosbati</i> , dead leaves.	Bethel, Vt.	Sept. 25	Sept. 26	0
<i>R. rotundifolium</i> , vigorous leaves.	No. Hudson, N. Y.	Sept. 29	Oct. 1	x
<i>R. nigrum</i> , vigorous green leaves.	Wilmington, N. Y.	Sept. 28	Oct. 7	xx
<i>R. nigrum</i> , fallen, dead, and dying leaves.	Wilmington, N. Y.	Sept. 28	Oct. 1	0
<i>R. nigrum</i> , dead leaves on plant.	Wilmington, N. Y.	Sept. 28	Oct. 1	(x)
<i>R. cynosbati</i> , vigorous leaves.	Bethel, Vt.	Nov. 12	Nov. 12	xxx
<i>R. cynosbati</i> , dead leaves under snow.	Bethel, Vt.	Dec. 1	Dec. 1	0
<i>R. cynosbati</i> , leaves killed by frost and lying on snow.	Bethel, Vt.	Germinated naturally xxx Dec. 3		

\*xxx Maximum germination

xx Medium germination

x Light germination

(x) Very slight germination

0 No germination

*Ribes* tested, yet leaves of *R. cynosbati* which remained upon the bushes until December, although frozen, yielded strongly germinating telia,

as late as December 3. (See Tab. 2.) Such leaves were collected which had dropped to the surface of the snow a short time before and the telia thereon had germinated naturally with maximum vigor. It was noted that diseased leaves of *R. nigrum* and *R. cynosbati* when dried almost invariably rolled their edges upward so that the entire lower surface of the leaf was exposed, thus insuring a maximum distribution of sporidia of the telia thereon.

Since early in the investigations of this fungus and the disease caused by it, it has been recognized by many investigators that *R. nigrum* is far the most dangerous species of *Ribes* known. It is dangerous as compared with other species because: (1) The plant is nearly maximum in height. (2) It is maximum in vigor of growth. (3) It produces new growth throughout the season. (4) It produces new shoots and leaves to a maximum lateness in the season. (5) It produces a maximum area of leaf surface. (6) It is more susceptible than any other species. (7) A maximum number of telia per unit of leaf area are produced upon this host. (8) These telia are of maximum vigor in germination and production of sporidia. (9) As shown by the above investigations, the telia produced on this host are of maximum longevity in the season. The actual danger from *R. nigrum* varies greatly in different localities because of the great variation in the number of bushes that have been planted there. One region, like the infected section of Washington, will have relatively large numbers, while other places, like the Adirondacks, will have very few or none.

Finally, attention should be called to the fact that these investigations show that telia upon *Ribes nigrum* and, to a lesser extent, upon *Ribes americanum* and *Ribes cynosbati* will remain alive in the winter and will germinate readily when the temperature rises a few degrees above freezing, as shown by the natural germination of telia on *R. cynosbati* leaves on December 3 when lying on the snow. The danger of infection of pines, as indicated by the presence of germinable sporidia, extends indefinitely into the winter in northern New England. It remains to be determined, however, whether the pines are in such condition at this time of year as to become infected.

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# INSECTS AS DISSEMINATORS OF PLANT DISEASES.

## I. RESULTS OF PAST INVESTIGATIONS<sup>1</sup>

FREDERICK V. RAND

I am unable to disclose anything particularly new at this time since my remarks are limited to the results of past endeavor. However, some of the facts to be presented, though not new, are none the less startling in their import. Just thirty years ago, in the year 1891, Waite gave the first demonstration of the insect dissemination of any disease, plant or animal, caused by micro-organisms when he showed that in nature the spread of the bacterial fireblight of pomaceous fruit trees is brought about almost exclusively through the agency of insects. Two years later Theobald Smith and Kilborne put forth their experimental proof that Texas fever is transmitted by the cattle tick (*Ixodes bovis* Riley); and soon thereafter Erwin F. Smith gave experimental evidence of some of the insect relations of several diseases of plants, bacterial in their nature. During the approximately three decades following these early endeavors the interrelations and correlations between pathogenic micro-organisms and insects for the spread of disease have received steadily increasing attention at the hands both of plant and animal pathologists and entomologists.

It is difficult to give in a very limited space any adequate conception of the mass of evidence thus far accumulated as showing the really important rôle played by insects and certain small arachnids either in the direct distribution of the micro-organisms of plant disease, or in the providing of wounds for their ready entrance into the host tissues. Two years ago, in preparing the manuscript, "A Coordination of our Knowledge of Insect Transmission in Plant and Animal Diseases," by Rand and Pierce (later published in *Phytopathology* 10: 189-231, 1920),<sup>2</sup> we attempted to classify the different types of insect transmission. This we did with the full knowledge that here as elsewhere in biology no hard and fast lines can be drawn. However, several distinct methods do exist and we believed that such a classification would serve a useful purpose in the mental grouping of insect relations to pathogenic diseases.

In the dissemination of the bacterial fireblight of apples and pears by bees or of the fungous internal boll disease of cotton by several sucking insects the causal organisms are carried in or on the mouth parts or other external portions of the insect body and are directly inoculated into the plant host under conditions suitable for parasitic growth. In some cases of this kind there is a distinct insect adaptation, beneficial to the plant parasite, although the insect concerned is in no sense an intermediate host or reservoir for the parasitic organism. On the other hand, in the dissemination of the bacterial heart and rib rot of cabbage caused by *Bacterium campestre* (Pam.) EFS by flies and in a large part of the spore dispersal by various insects in the case of the chestnut blight fungus the organisms, carried in a viable condition mostly on the insect bodies, are accidentally sown rather than directly inoculated or "put into" the host tissues.

<sup>1</sup> Paper read at the joint session of the Phytopathological Society and the American Association of Economic Entomologists, Toronto, Canada, December 31, 1921.

<sup>2</sup> The writer has in preparation a review which will bring this paper up to date. Separates and suggestions will be appreciated.

In other cases, as in the bacterial boll rot of cotton and in many of the chestnut blight infections the role of insects consists in the provision of points of entry for infective material brought there by other agencies than the particular insect making the wounds.

In still other instances, as in the *Leptosphaeria* fungus causing apple canker and raspberry cane blight, the spores are carried not only superficially but remain viable after passage through the digestive tract of the insect. Although the relation here is merely a mechanical one there is nevertheless thus afforded a double chance for infection to be effectively brought about.

However, the clearest cases of adaptation for disease transmission are found where the infective principle obtains within the insect body conditions favorable for further development or multiplication. Among bacterial diseases of plants such a relation has been shown to occur between the olive-knot organism and the olive-fly (*Dacus oleae* Rossi) as worked out by Petri, and between the bacteria of cucurbit wilt and the striped cucumber beetle as shown by my own work in collaboration with Enlows and Cash.

In correlating the published and unpublished data at hand relative to cucumber wilt the seasonal history appears to be as follows: A small percentage of striped cucumber beetles come out of winter quarters as "wilt carriers." As they feed upon young cucumber seedlings their feces laden with wilt bacteria become mixed with dew or light rain and are thus brought into the intimate contact with fresh injuries opening into the vascular system which are necessary for any infection to take place. In this manner originate the sporadic primary cases of the disease seen in early spring. Once started in this manner the disease is then largely spread from plant to plant through mechanical dissemination by the mouth parts of the voracious and actively flying beetles; and the rise in the disease curve corresponds closely with the increase in the prevalence of these insects during the advancing season. Dissemination by two species of *Diabrotica* constitutes the only known method by which this disease is spread in nature.

In the various infectious chloroses of plants we have a whole train of diseases or related groups of diseases in which the insect relation is clean cut and often specific. As shown by Ball and later corroborated by others the beet leafhopper (*Eutettix tenella* Baker) is not only the sole means of transmission for the disease but it appears from the work of R. E. Smith and Bonquet that at least twenty-four hours must elapse after feeding on a diseased plant before the insect is capable of giving infection. Carsner and others have demonstrated that this disease is readily intercommunicable as carried by leafhoppers among a considerable number of other cultivated hosts and wild plants. McClintock and Loren B. Smith have transmitted spinach blight by the tarnished plant bug and by three species of aphids, and have carried the infective principle through four generations of aphids after one feeding of the original parents on blighted spinach. Similar transmission of mosaics or other chloroses of potato and tobacco, of the cabbage and clover families, of sugar cane and other members of the grass family, of red raspberry and many other hosts and groups of hosts are now familiar to all workers on this type of disease. Doolittle and his collaborators have shown that an important part of cucumber mosaic transmission is not only through the agency of sucking insects but also by means of two species of cucumber beetles. Furthermore, through the use of one or the other of these insects he has carried over the malady not only to various wild and cultivated members of the cucurbit family but apparently also to pokeweed and potato.

TABLE 1  
*Insect dissemination of plant diseases<sup>1</sup>*

Mode of dissemination	Bacterial			Fungous			Pro- to- zoan	Filter- able con- tagium	
	Experimental proof	Strong circum- stantial evi- dence	Doubtful	Experimental proof	Strong circum- stantial evi- dence	Doubtful		Experimental proof	Strong circum- stantial evi- dence
External dissemination and direct inoculation	6		1	3	9			(1)	
External dissemination without direct inoculation	(1)	3		4	8 (1)	1			
Wound infection from other sources than the wounding agent	1	3		2 (1)	6				
Internal mechanical (unchanged passage)				5	2				
Internal biological (Multiplication within carrier)	2	1		2			1	59	7
	9	7	1	16	25	1	1	59	7

<sup>1</sup> Figures in parentheses indicate that the disease has also been enumerated under another type of insect dissemination.



This is hardly the place for a discussion as to the nature of the causal agents in these diseases or of their relations to each other. However, the similar sequence of symptoms, both external and internal, and the progressive connecting up by different workers of various groups of host plants among which mosaic is intertransmissible surely suggests a probability that the causal agents of infectious mosaics are closely related. Furthermore, in some cases at least, the data at hand suggest that the insect may be not merely a mechanical carrier but should be considered as a true intermediary host of the infective contagium.

Odious though statistics often may be, perhaps the clearest way to show briefly what has been done by entomologists and pathologists relating to insect dissemination of plant diseases is by means of a numerical summary. Using the data which I have gleaned from the literature a table has been prepared showing the number of diseases, —bacterial, fungous, protozoan and filterable contagium,—carried by the different types of insect dissemination. The figures are not to be considered as absolute since new investigations will, of course, continually change our outlook, and some work already published may have been overlooked. For full proof of insect dissemination in any specific case I have considered it necessary to show that under natural conditions the insect is in the habit of visiting both diseased and healthy plants, of carrying the causal contagium and depositing it in places favorable for infection, and that infections actually do thus result in nature. If any of these steps have been omitted, even though the evidence is rather clearly incriminating, I have placed the disease under the "circumstantial evidence" column in the table.

To summarize briefly (cf. Tab. 1), then, full proof is forthcoming for nine bacterial diseases. This is followed by strong circumstantial evidence in seven other cases and rather doubtful evidence in the one remaining case. This represents sixteen or seventeen bacterial diseases in which insect dissemination in one form or another plays a part. Among fungous diseases of plants the proof is definite for sixteen diseases, followed by strong evidence for twenty-five and doubtful evidence for one—or a total of forty-one or forty-two. In one protozoan disease, a flagellosis in the latex of certain Euphorbias (caused by *Leptomonas dividi* Lafont), Lafont has shown the sucking insect, *Nysius euphorbiae* Horvath. to be a true intermediate host. Most of the flagellates of this group are normal inhabitants of insects.

In the case of the mosaic group of diseases I have found fifty-nine hosts for which insect transmission has been fully demonstrated and seven where the circumstantial evidence is strong, making a total of at least sixty-six hosts where insects spread mosaic.

Among the bacterial and fungous diseases of plants insect dissemination, where it occurs, is in some cases sporadic and incidental. In a considerable number of these diseases, however, as for example, in the fireblight or in the English blossom blight of pomaceous fruit trees and in cucurbit wilt among bacterial diseases, and in the cotton internal boll disease, carnation bud rot, pineapple Thielaviopsis, chestnut blight, apple Leptosphaeria canker, corn stalk and ear molds and many other fungous diseases, insects bear a really important relation to the spread of disease, either directly as actual carriers of infection or indirectly by their provision of wounds through which the organisms may enter. It is, however, among the infectious chloroses, including the mosaics, that we find whole groups of diseases where insects appear to be the principal and in some instances the only means of spreading the disease in nature. Their importance in this connection, therefore, can hardly be overestimated.

## INSECTS AS DISSEMINATORS OF PLANT DISEASES.

### II. SYSTEMATIC RELATIONS OF CARRIERS<sup>1</sup>

E. D. BALL

Mr. Rand has given you a resumé of the whole subject. I only want to add to that a little analysis of the insects included. This may be more for the benefit of the pathologists. A study of a large number of these insect disseminated diseases will develop the fact that the insect contact is entirely accidental. That is, any insect that lights on a pear tree and sucks the juices may carry pear blight, and a large number of cases occur in which there is no adaptation on the part of the insect, or any peculiar type of insect required for this distribution.

When you come to the cucumber mosaic, and the cucumber wilt, the carrying over winter of this disease by the beetle infers something a great deal more definite and important than the other type of transmission. There must be a multiplication of the bacteria in the intestinal tract or in the body cavity (the body fluid) of that insect to maintain this disease in a viable state for so long a period of time.

The actual transmission to the plant is shown to be entirely accidental, due to the excreta being distributed over the tissues of the plant and coming in contact with the cut surfaces made by the feeding of the beetle, but we must have a conception of an adaptation of this germ to a life in the insect during the hibernating period, and even longer than that.

That opens up a new field and you will note there that the cucumber beetle is a leaf feeding, a biting insect, but that the transmission as far as the mechanical part of the insect is concerned, is entirely accidental. It is not transmitted apparently through the mouth, but through the feces.

In Rand's second division we get to a relation that is obligatory. In the case of the Euphorbia plant the little false chinch bug which is a sucking insect deposits saliva in the wound, and with it one stage of the organism. There is a stage of the development of the protozoan parasite in the insect and another in the milky juice of the Euphorbia—a typical case of a transmission of a plant disease in exactly the same way that a large number of animal and human diseases are transmitted. That suggests the possibility that we will find a number of protozoan diseases in plants before we are through.

When we come to the curly top of the sugar beet—there we get a transmission which is absolutely specific again, but there is only a single species of insect (though there are a number of that same group of insects that feed on sugar beets) that is able to carry that disease over winter, and reproduce it on the sugar beet. Nothing more seems to be known about what it carries or how it is carried except that he is not able to transmit the disease immediately after he has punctured a diseased beet. It takes an incubation period usually of two or more days. That may be entirely mechanical. It may be simply the time required for the passage from the alimentary canal back through the salivary glands, and to a point where it can be passed to the new plant. Many of the facts indicate an incubation stage in the insect such as we find in protozoa parasites.

<sup>1</sup> Paper read at the joint session of the Phytopathological Society and the American Association of Economic Entomologists, Toronto, Canada, December 31, 1921.

The fact that the leaf that is punctured on the sugar beet is not the one that shows the disease, but the leaf that is growing, shows that whatever is injected into the sugar beet passes down into the beet root itself, and then back up into the leaf.

Not only that, but if you cut all the leaves off the beet, the leaves that come up again will be diseased. If you silo the beet over winter and grow a seed beet next year, it will be diseased, showing that the whole organization of the sugar beet has been infected, and a rather rapid transportation of that infection from part to part of the sugar beet.

When you come to the tipburn or hopperburn of the potato you get a different situation, for there is no movement of the disease in the potato. The leaf that is attacked is the one that is injured, and no other. The disease is not in any sense progressive. The leafhopper that attacks a single leaf has no effect on any other leaf of the potato, up to the time of the death of the plant.

I have not been able to take the time to trace out the relationships in this newest of our discoveries on the cane mosaic. The spinach mosaic does not appear to be specific. There are two species of plant lice and one of tarnished plant bug that have transmitted this disease. So that leaves that in a doubtful category at this time.

It may be interesting for you to note one thing about these insects. I know nothing at all about the life history, except in a general way, of the false chinch bug. Let us take the two diseases we have here—the beet leafhopper and the potato leafhopper. There is at once a remarkable thing about both of them. The insects winter over as adults in both cases, and that is rather exceptional in leafhoppers. The females that pass the winter live until late in the summer and some of them to the frost period in the fall. Dr. Fenton and Mr. Hartzell kept one of these insects until September 7th; that was undoubtedly developed as an adult during July or August of the year previous. This particular insect laid eggs up until almost the end of that period, laying one hundred forty-two eggs, an average of one egg a day.

The potato leafhopper does not have time to complete the second brood under the climatic conditions of central Iowa. The beet leafhopper in the regions where I studied it, is a single generation insect, and normally the great majority of those remain in the sugar beet field until very late in the season, laying eggs through a period of time long enough for three generations of the insect to have matured if it had an ambition to have numbers of generations.

The same thing is true of the potato leafhopper. Just exactly what relation this extreme length of life has to the problem is not quite clear. The fact that it lives over winter as an adult may be necessary in order that it carry the disease over winter. In other words, it does not, in the case of the beet leafhopper, transmit this disease through the egg. If you take a beet leafhopper as it comes out of the egg, before it has had time to turn around and suck the diseased plant from which it was hatched it does not carry the disease.

In hunting for the solution of these problems it appears that we ought to keep in mind the fact that the chances are very great that the insect that transmits any one of them will be one that hibernates as an adult; that in a case of specific transmission it will probably be a sucking insect, and an insect with a long adult life. The reason for this last conclusion is not clear.

There is, of course, the possibility that under wild conditions with a large number of generations, that a generation might be compelled to migrate to a plant that did not carry the disease, and then the generation that came back would be without it. Unless the disease is of some advantage to the insect there is no reason for that inference, but in the case of the beet leafhopper the disease is apparently an advantage to the insect. The beet leafhopper has a half dozen relatives that produce the same

type of injury on the sunflower, the pigweed and other related weeds, and in every instance, the color of the malformation on the leaf is the color of the larvae of the insect and this color together with the curling serves as a protection.

The potato leafhopper is a native of South America, but has spread to North America and is now appearing in Europe so that tipburn may be expected to spread to other regions. The beet leafhopper is a native of the arid southwest and the curly leaf is at present confined to that region.

I have occupied more time than I should in bringing out some of the points that we should keep in mind in studying this problem from the insect standpoint. (Applause.)

## INSECT AS DISSEMINATORS OF PLANT DISEASES. III. CONTROL PROBLEMS<sup>1</sup>

L. CAESAR

I have been asked by the committee in charge to introduce the control problem aspect of this morning's program and do so with much diffidence, partly because of lack of sufficient familiarity with the diseases and partly because of pressure of other duties which have prevented my giving the thought to the subject that it required. I shall not attempt to outline methods of control for any disease but will mention some of those proposed by different persons or that have suggested themselves to me, then refer to some difficulties that beset control and finally discuss the insect aspect of the problem.

The following are the general recommendations for control as outlined by investigators or as inferred from what we know of the life histories of the diseases: First, remove and destroy promptly all infected plants beginning as early in the season as the symptoms of the disease disclose themselves. Second, ward off or destroy insect disseminators. Third, take the necessary precautions against spreading the diseases during the growing season by mechanical means such as pruning, harvesting and careless cultivation. Fourth, destroy at once after harvesting the crop all remnants, if there is any suspicion that these may be a source of danger. Fifth, select seed wherever possible from plants known to be healthy. (Do the same with tubers in the case of potatoes.) Sixth, endeavour to find and remove the source of primary infection in spring whether it be other host plants of the disease, especially perennial weeds, or some other source. Seventh, take precautions to secure and maintain healthy seedlings in spring. (This is more or less covered by some of the preceding.) The securing of healthy seedlings or other sources of plants such as tubers may of course involve special isolated seed plots, carefully inspected and protected throughout the season. Eighth, the discovery and selection where possible of immune varieties.

Now let me call your attention to some of the great obstacles to carrying out the above. First, we have not sufficient knowledge of the relationship between the different diseases themselves (I refer particularly to mosaic diseases). For instance, is the mosaic of potato a distinct disease from that of tomato and of cucurbits? This is an exceedingly important matter and there still seems to be some doubt about it. Second, we evidently do not yet know all the wild host plants of the different diseases, more being

<sup>1</sup> Paper read at the joint session of the Phytopathological Society and the American Association of Economic Entomologists, Toronto, Canada, December 31, 1921.

found every year. Not to know these is to be greatly hampered in finding control measures, especially as perennial weed hosts may serve as a method of overwintering of the disease and thus as the primary source of infection in spring. Third, in a few cases there still seems to be some doubt whether certain of the diseases may not overwinter in the seed bed or in the soil and become the primary source of spreading the disease in spring. Lastly we need more definite knowledge of the cause or causes of the great fluctuations of some of the diseases from year to year. The absence or presence of insect disseminators does not appear to be the solution of these fluctuations, for instance, many of us have observed the remarkable absence of potato mosaic some years and its prevalence other years. Professor Howitt tells me that so far as his experience goes mosaic is every year present and its symptoms quite evident in northern Ontario. What is the explanation? Is it that the disease is present in southern Ontario and other places annually but that some years its symptoms are suppressed, or is there some other factor that we have overlooked?

From these and other considerations it is evident that the greatest aid to the final solution of control problems lies in further life history studies. This, of course, is no justification for our not trying in the meantime whatever control measures appeal to our judgment and securing from them what light we can.

In turning to the insect aspect of our subject we are all agreed I suppose that by warding off the insect transmitters the control of the diseases in question would be made much simpler and perhaps in the majority, or possibly all, of the cases solved. But satisfactory insect control is with a few exceptions so difficult and so costly that it is doubtful whether in the long run we shall not have to find some other way of accomplishing our purpose. The only insect-produced disease that I can think of at present that has been simply and satisfactorily solved by insect control is the hopperburn of potatoes. This disease is of a simpler type than mosaic or any of the others. Bordeaux mixture acts as a repellent for the leaf hoppers and thus protects the plants, and, as Bordeaux is used in any case in good potato culture, the extra cost of controlling the insects is very little.

The striped cucumber beetle is apparently the sole transmitter of cucumber wilt and an important agent in the spread of the mosaic on cucurbits. Control measures for this insect as known at present are merely helpful and far from satisfactory. Our duty here evidently is to begin a new study of the insect and discover if possible a thoroughly satisfactory means of combating it.

Several species of aphids are the main agents in transmitting the various mosaic diseases, leaf roll, raspberry leaf curl and spinach blight. The control of these aphids is rendered very difficult, especially some years, by the following obstacles: First, they are marvellously prolific: scarcely any other kind of insect can compare with them. At first there may be only a few scattered individuals in the plot but in a few weeks these sometimes will have increased to millions. Second, most of the species involved have many host plants; for instance, the peach aphid, *Myzus persicae*, and the potato aphid, *Macrosiphum solanifolii* have each more than fifty different host plants, most of them being weeds, and yet these are two of the most important aphids involved in the diseases referred to. Hence, while we are trying to protect a certain kind of plant these aphids may be breeding everywhere in the surrounding vicinity. Third, throughout most, or in some cases almost all, of the growing season there are both winged and wingless forms of the insect. The wingless forms travel slowly but the winged forms, one of whose functions seems to be to distribute the species, move freely from place to place; so that no matter how clean a plot may be one week it is likely to be reinfested the next. Fourth, aphids have the habit of rolling leaves and working under the protection of the rolled area where it is very difficult to reach them

with spray mixtures. This makes treatment slower and more costly. Fifth, sprays for aphids are of the contact type, killing either by hitting or by the fumes given off, hence their effect lasts only for a few days at the most, this means that they have to be repeated frequently. Moreover, nicotine used either in the liquid or dust form is the only efficient insecticide at present available and is very costly, hence numerous applications of it would mean that the grower in at least many cases would prefer to give up the crop rather than face the trouble and expense.

Such then are the difficulties involved in the control of the aphid transmitters. All of these difficulties, however, are not always active and so the task sometimes is not nearly so hard as it looks. For instance, the following favoring conditions sometimes act on our side and render control practicable: First, in a hot season the aphids usually disappear, or almost disappear, during July and August. Second, some years the various factors that help to control aphids are very powerful throughout the whole season and so reduce their numbers that very few are present at any time. Third, some of the plants in question, as for example, tomatoes and tobacco, are not as a rule favorite hosts of aphids. Lastly in the case of raspberry leaf curl, transmitted by *Aphis rubiphila*, as discovered by Dr. Rankin, the insect under field conditions seems to be wingless and to move about very little. Dr. Rankin has found that as a result of this he has been able by early roguing of infected plants to control the disease without having to fight the aphids.

In conclusion, without discussing the tarnished plant bug, which is the other main offender and for which we have no satisfactory control, I shall merely call attention to the fact that the study of the relation of insects to these diseases has emphasized our need of an efficient and cheap contact insecticide for aphids and other sucking insects such as leaf or plant bugs.

## INSECTS AS DISSEMINATORS OF PLANT DISEASES.

### IV. URGENT PROBLEMS OF THE FUTURE<sup>1</sup>

MAX W. GARDNER

In order to be able to call to your attention some of the really urgent problems of the future in connection with insects as disseminators of plant diseases, I have made an effort to ascertain the opinions of a number of pathologists and entomologists who have conducted research along this line and have attempted to correlate and summarize the information thus acquired.

All of us are, I assume, primarily interested in being of real service to the growers of agricultural crops, in increasing the production per acre, and last but not least in serving science. I choose to avoid further generalities and to be as specific as possible in the short time allotted.

I believe I am safe in asserting that comparatively few types of plant disease are more or less strictly dependent upon insects for their dispersal. From an economic point of view based upon the importance of the crop and the prevalence and destructiveness of the disease, I believe we can safely eliminate from this category practically all of the fungous diseases and all but a few of the bacterial diseases and concentrate our attention upon the really important insect-borne diseases. Permit me briefly to dispose of two outstanding cases among the bacterial diseases, namely, fire blight of apples and pears and wilt of cucumbers and cantaloupes.

<sup>1</sup> Paper read at the joint session of the Phytopathological Society and the American Association of Economic Entomologists, Toronto, Canada, December 31, 1921. Contribution from the Botanical Department, Purdue University Agricultural Experiment Station, LaFayette, Indiana.

Smith (31 p. 215) and Rand and Enlows (25) have shown that cucumber wilt is strictly dependent upon the striped and spotted cucumber beetles for both dissemination and overwintering of the pathogene. Here, it would seem, pathologists have done all within their power for the present, all further progress being dependent upon the development of a more perfect control of the two species of beetles.

The fire-blight situation is more complex. Recent work in Ohio (15) and in Illinois (34) on rain drip and wind-blown-rain infection and by Heald (14) on leaf water-pore invasion indicates that possibly too much emphasis has been placed on the insect relations. The whole situation should receive further study by pathologists with a view to the improvement of blight-control by some such measures as spraying and the development of resistant varieties. The root and collar blight in the Northwest and the mode of transmission from such lesions to the blossoms presents a problem to both entomologists and pathologists.

There remains for our consideration a rather homogeneous group of very important plant diseases, largely insect-disseminated, and I feel certain that I am correct in assuring you that by far the most urgent need of the present is a better understanding of this group of diseases—the so-called virus or mosaic diseases. Dr. Rand and Dr. Ball have just explained how closely these diseases are associated with certain insects as disseminating agents. When one considers the large number of important crops subject to one or another of this type of disease, and the extreme destructiveness, the rapidity of spread, and the apparent annual increase in the prevalence of these mosaic diseases, their importance can hardly be overestimated. I know of no diseases more baffling and discouraging to the grower and the pathologist.

To attempt, in the time allotted, to give an account of all of the urgent needs in connection with our fragmentary knowledge of these troubles is impossible. This group of diseases offers not only a challenge, but a rare scientific opportunity because of the similarity of the symptoms and behavior in the many different hosts. An advance in the understanding of any one of the mosaics is very likely to throw much needed light upon all the rest. Our understanding of this group of insect-borne diseases stands in the greatest need of painstaking and fundamental research by both pathologists and entomologists.

The investigation of mosaic diseases necessitates a specialized technique which in itself makes it essential that the entomologist and pathologist work in close association in the solution of certain phases of the problem since either one working alone may err seriously. Such investigation also necessitates expensive equipment and the desirability of enabling isolated workers with poor facilities to take their problems to a well-equipped institution at the proper time should be recognized.

I desire to illustrate by specific examples some of the urgent problems presented by the mosaic diseases and I have attempted to arrange these problems in three classes, first, those which it would seem to be the duty of the pathologist to solve, second, those which must be solved by the entomologist, and third, those which will very likely necessitate the combined efforts of pathologists and entomologists. It should be remembered that for certain mosaic diseases some of these problems have already been at least partially solved.

The problems for which the pathologists alone are primarily responsible may be grouped under five headings, as follows: 1. The nature of the mosaic contagium, infective principle, or virus. 2. The physiology of infection. 3. Transmission by other means than insects. 4. Host range of each virus, with especial reference to wild carriers of infection. 5. Development of disease-resistant varieties.

With respect to the nature of the contagium or virus, a number of methods of attack are open which may help us to ascertain whether the causal entity is a lifeless chemical compound or an organism.

By filtration through bacteria-proof filters it has been proved that the causal entity in several mosaic diseases is not of the nature of ordinary bacteria. Allard (3), Doolittle (8, p. 24), and others have shown that the viruses of tobacco and cucumber mosaic will pass the coarser filters such as the Berkefeld type but not the finer filters such as certain Chamberland filters, atmometer cups, and talc layers. Since the viruses of certain human and animal diseases not only pass these finer filters but collodion filters as well, it is evident that the causal entity of plant mosaics is not as finely divided as that of the animal filterable virus diseases. By careful differential filtration with several types of atmometer cups in comparison with certain chemical compounds, Duggar has recently been able to arrive at a close approximation of the size of the active particles in the tobacco mosaic virus.

By the effect of dilution of the juice of a mosaic plant upon its infective power, some idea of the concentration of the causal particles can be obtained. Allard (1) working with tobacco mosaic and Doolittle (8, p. 21) working with cucumber mosaic have found that a dilution of 1:10,000 was about the upper limit at which any infectivity was retained. Animal pathologists report that animal viruses retain their infectivity in very much higher dilutions. The fact that in the case of cucumber mosaic the incubation period is no longer with the diluted than the undiluted juice and that within four days the plant may be completely permeated with the virus indicates a most remarkable power of multiplication and diffusion on the part of the causal entity.

By the use of the ultramicroscope, the range of which extends downward to diameters of about six one-thousandths of a micron, upon the infective filtrates, light may be shed upon the nature of the particles.

Careful cytological study of diseased host cells should yield extremely interesting information. Iwanowski (18) working with tobacco mosaic and Kunkel (20) working with corn mosaic have found evidences of an intracellular organism. The microchemistry of diseased as compared with normal plants might yield instructive data.

The localization of the virus in the various tissues and organs of the host should be studied. Allard's work (2) on the presence of the mosaic virus in various organs of the tobacco plant furnishes an example of work of this character.

The effect of temperature, storage, desiccation, and various hydrogen-ion concentrations upon the virus in vitro and the effect of various reagents and germicides may yield important information concerning the causal factor. The investigations of Allard (3, 4) and later of Doolittle (8, p. 19) along this line have been very suggestive.

Attempts to culture an organism from the virus have only begun. Only a limited range of media has been tested. The possible significance of specific compounds and accessory factors should be considered. Variation of the oxygen and carbon dioxide pressures and of the H-ion concentration in a wide variety of media, including host tissue preparations, might yield interesting results. The strikingly beneficial results of an increased CO<sub>2</sub> tension (17) on the growth of certain animal pathogenes and of a decreased oxygen pressure in the culture of certain human pathogenes is of interest in this connection. The success reported by Flexner and Noguchi (13) with the culture of the virus of poliomyelitis should encourage the plant pathologist.



Duggar (10, p. 288) has suggested that delicate tests should reveal the presence of life activity in the virus in vitro, if it is an organism comparable to any types of which we now conceive.

The work of Löhnis and Smith (21) on the life cycles of certain bacteria and the existence of pleomorphic species is highly suggestive in connection with filterable viruses in general. Rosenow and Towne (28) report that the streptococcus held by them to be responsible for poliomyelitis may under certain conditions become sufficiently reduced in size to pass the filters.

The physiology of infection should be studied with such points in mind as:

1. The effect of age of host, temperature, humidity, and soil conditions on susceptibility, incubation period, and severity of the disease. Recent work by Johnson (19) and by Doolittle on the effect of temperature has yielded striking results.

2. The possibility of the isolation or development of resistant strains or varieties of the host. The resistance of the bean variety known as Robust Pea to mosaic, the development of a mosaic-resistant variety of spinach by Smith (33) at Norfolk, and the discovery (29, p. 28) of a mosaic-resistant strain of the Bliss Triumph potato indicate that resistance may be found in other crops subject to mosaic diseases.

3. The relative susceptibility of certain organs and tissues. In some cases the phloem tissue appears to be particularly susceptible to infection.

To the pathologist falls the study of the mode of transmission by agencies other than insects, by such operations as artificial pollination of greenhouse crops, cultivation, pruning, and picking, and the study of dissemination by the transport of seed or reproductive parts such as tubers and cuttings, of nursery stock, and of transplants. The possibility of mosaic transmission in the pollen has been suggested. The likelihood of control by roguing early sources of infection should be ascertained.

The possibility of the overwintering of the virus in the seed (as in bean and soybean mosaic) or vegetative reproductive parts (as in potato leaf-roll and mosaic and sugar cane mosaic) of the host and how to produce or obtain disease-free seed are the pathologist's problems.

Host range studies are of basic importance. This applies to cultivated crops as well as wild hosts. Mosaics enphytotic in wild hosts may be the original source of these crop troubles and all mosaics in weeds or wild plants should be viewed with extreme suspicion. The question of wild perennial hosts is of vital importance in connection with the mode of overwintering of the virus in the mosaics of certain crops. For example, the perennial ground cherries and horse nettle constitute a reservoir of mosaic infection for the tomato crop (14) and Doolittle and Walker (9) have found that milkweed perpetuates the cucumber mosaic virus. Carsner (7) has found that a winter annual weed (*Erodium cicutarium*) in California carries the beet curly-top virus. The mosaic symptoms may be obscure or lacking in certain of these wild carriers of infection especially in old plants late in the season. In the cases of cucumber mosaic and tomato mosaic, extensive field observations indicate that, once introduced among the perennial weed hosts in a locality, the disease persists year after year and, as new fields are used for the host crops, the reservoir of mosaic infection in the perennial weed flora increases annually. In fact this reservoir of infection may increase at an alarming rate and, as in the case of cucumber pickle growing, drive the industry out of one region after another.

For the solution of many very important questions we are dependent entirely upon the entomologists. Speaking for the pathologists, I may say that in many instances further progress in mosaic control rests wholly with the entomologists. In fact I be-

lieve an appeal should be made to entomologists in general to acquaint themselves with and interest themselves in this group of complex insect-transmitted diseases. Furthermore, pathologists must depend upon entomologists for an explanation of the true nature of such troubles as potato hopperburn which are of insect causation. It is with great hesitation that I venture to make suggestions in a field of science other than my own, but certain needs are outstanding.

In many cases we know that mosaic transmission is accomplished entirely by certain species of aphids or leafhoppers. The obvious necessity in these cases is a really effective control for these particular species, not the degree of control which would be considered satisfactory from the standpoint of actual insect damage, but an absolute control, because one aphid or leafhopper alone may be able to inoculate a plant.

Might not more detailed study be concentrated upon the particular insect species already incriminated as carriers? Such a study should disclose important features in connection with the life history of these carrier species under the particular local conditions where the disease occurs, such as the effect of temperature, humidity, and season upon life cycle changes and general activity, the maximum longevity of individuals, the possible distances which individual carriers may travel, and the complete list of food plants and the plants preferred by each species under various conditions of season and locality with especial reference to wild mosaic hosts. The importance of these points in relation to the inception and severity of mosaic epiphytotics cannot be over-emphasized.

Carsner has suggested the term, "viriferous," to characterize the virus-bearing individuals among the insect population. Eventually we should know the histology and physiology of viriferous as compared with non-infective individuals of the carrier species. Does the insect function as an alternate host for the pathogene? Is the viriferous insect diseased?

A more intimate and fundamental understanding of the relation of carriers to the plant tissue and to symptoms produced is essential. The type of tissue punctured or injured and the constancy of feeding or ovipositing habits are important points. Why are aphids the surest means of securing inoculation with certain mosaics? Is it because they habitually penetrate a highly susceptible tissue such as the phloem?

The fact that in Europe the term, phytopathologist, is applied to both pathologists and entomologists bespeaks a point of view based primarily upon host injury and host reaction rather than the classification of the causal factor concerned. Such a point of view is much to be desired. Similar symptoms may be brought about by different causes. The work of Ball (6) and of Fenton (11) has clearly demonstrated the cause of hopperburn of potatoes and undoubtedly there is much to be learned about similar troubles in other hosts.

With insects as well as parasitic microorganisms the exact cause of host injury should be ascertained and proved somewhat in accordance with Koch's rules of proof. Smith (32) in England recently reported on his studies of the injury produced by a certain plant bug on apples. He recognized that the lesions might be due to laceration alone, to some toxic secretion of the insect, or to a transmissible parasitic organism and by inoculation and cultural tests he proved that the lesion was the result of a toxic salivary secretion. Recent work by Fenton and Ressler (12) on artificial production of potato tipburn with an emulsion of crushed adult leafhoppers also is illustrative of the type of work desired.

The possibility that certain strains or varieties of the host plant may prove somewhat resistant or distasteful to the particular insect carriers involved is indicated by Smith's observations (33, p. 154) on spinach mosaic in which he found the varieties Manchuria and Virginia less subject to aphid infestation than the Savoy variety.

The third class of problems are those to be solved preferably by some form of collaboration between entomologists and pathologists. A number of these problems have been outlined by Rand and Pierce (27, p. 220).

The determination of the particular species of insects involved in the transmission of each mosaic disease is essential. This necessitates actual transmission under controlled conditions and applies not only to the cultivated crops but the wild host plants as well. The relation of season and geographical location to the carrier species involved may be important. Particular attention should be given to the insects common to both cultivated and wild host plants, especially those present early in the growing season. Much remains to be determined about the natural transmission of many mosaic diseases such as bean mosaic, tomato mosaic, soybean mosaic, clover mosaic, potato leaf-roll, peach yellows, and pecan rosette. With the mosaic disease of potato the possibility of transmission by underground agencies is suggested by Quanjer's results (24), while Schultz and Folsom (30) have found no evidence of such a phenomenon. What is the fundamental difference between beet curly-top which is successfully transmitted only by the one species of leafhopper and such virus diseases as cucumber mosaic which may be transmitted in several ways? The relationship of the beet curly-top of the west to somewhat similar beet diseases reported from other parts of the country is unknown as yet.

Once the agency of a particular species of carrier insect is established for the disease under investigation, many questions remain to be answered. Work of the character of that done by Ball (5) on beet curly-top, and by McClintock and Smith (22) on spinach mosaic illustrates the type of investigation necessary to answer some of these questions.

How long a period of feeding upon a diseased plant is necessary for the insect to become viriferous? Is there a necessary incubation period within the insect's body?

How long must a viriferous insect feed upon a healthy plant to cause infection? What is the minimum number of individuals that may cause infection? In some cases, one aphid has been found sufficient. What is the relation of this number to the incubation period of the disease in the plant?

How long, after feeding on a diseased plant, does an individual remain viriferous? This is important in connection with the perpetuation of the virus over winter or during the period when the crop is not being grown. Does the condition persist through metamorphosis? In what stages of its life cycle is the insect a carrier?

Is the viriferous condition or infective capacity transmitted to the offspring? Through how many generations may this occur? McClintock and Smith (22) found that one of the aphid species which transmit spinach mosaic retained its infective ability through four generations on lettuce.

Is infection caused by the bite or feeding puncture or by oviposition? What is the localization of the virus on or in the insect's body? Is it external as on the feet or mouth parts or is it in the saliva or alimentary canal contents? Petri's work (23) on the persistence of the olive-knot bacteria in the fourth gastric pouch of the olive fly and the work of Rand and Cash (26) on the persistence of the cucurbit wilt bacteria in the alimentary tract of the striped beetle are illustrative of the type of information desired. In the terminology used by Rand and Pierce (27), is the transmission mechanical or biological?

Why do various species differ in their transmitting ability? Is this correlated with feeding habit and type of tissue punctured or with inability of the virus to endure conditions in or on the insect?

For each mosaic disease such investigation may lead up to control by some such means as:

1. Use of varieties or strains resistant to the virus or to the insect carriers.
2. Adequate control of the insect carriers and other agencies of dissemination.
3. Elimination of original sources of infection by (a) use of mosaic-free seed, vegetative propagation parts, or transplants and (b) eradication of volunteer plants and wild hosts, especially perennial weed hosts, in and near fields, plant-beds, and hot-houses and maintenance of an isolation zone of a width to be determined by the distance traversed by the insect carriers.

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# THE COOPERATIVE POTATO SPRAYING PROJECT: REPORT FOR 1921

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WITH NOTES BY OTHER WORKERS

This brief report, to supplement the data given in *Phytopath.* 11, pp. 178-193 is made to bring the results of potato spraying and dusting experiments down to date. It is hoped that these experiments may be continued in the various regions where work is still needed.

*Manitoba.*—Preliminary tests were made by G. R. Bisby. Late blight has never been recorded in Manitoba, and early blight and tipburn (hopperburn) are not ordinarily serious. Spray and dust were applied in 1921 to a field of Bovee (Beauty of Hebron) potatoes at Winnipeg. The soil was not very good, and somewhat poorer toward one side; however, since the plots consisted of 4 rows sprayed or dusted alternated with 4 check rows, this source of error is compensated. A 5-5-50 Bordeaux was used, applied with a traction sprayer. Since the sprayer had but one nozzle per row, each plot was sprayed twice over at each application, the machine travelling in opposite directions. Three applications of spray were made July 16, 25, and August 4. Sander's copper lime dust was applied with a hand duster to the dust plots July 18, 27, and August 8. The sprayed plots were noticeably different during the summer, and remained green about 2 weeks longer than the check plots. No difference could be noted between dust and check plots. The potato beetle had been controlled on all plots with poison before spraying and dusting began. Some early blight and tipburn occurred, being worse on plots not sprayed. Yields were taken from the 2 middle rows of each plot.

TABLE 1  
*Yields in Manitoba in 1921*

Treatment	Rows No.	Ave. yield per acre bu.
Sprayed.....	2, 3	178.2
Check.....	6, 7	127.9
Sprayed.....	10, 11	170.5
Check.....	14, 15	152.1
Sprayed.....	18, 19	173.2
Check.....	22, 23	142.1
Dusted.....	26, 27	149.8
Check.....	30, 31	123.6
Dusted.....	34, 35	113.7
Check.....	38, 39	97.2
Dusted.....	42, 43	92.9
Check.....	46, 47	89.1

<sup>1</sup> Coöperative project of the Advisory Board of American Plant Pathologists.

We may summarize table 1 as follows:

Average yield of 3 sprayed plots.....	174.0 bu. per acre
Average yield of 3 adjacent checks.....	140.7 " " "
Average increase from spraying.....	33.3 " " "
Average yield of 3 dusted plots.....	118.8 " " "
Average yield of 4 adjacent checks.....	113.0 " " "
Average increase from dusting.....	5.8 " " "

These results indicate that spraying pays in Manitoba.

*Ohio*.—E. E. Clayton carried out an extensive series of coöperative experiments on potato spraying and dusting in Ohio during 1921. Forty-four spraying tests were made, principally on areas of 1 acre or one-half acre in fields in Cuyahoga and Lake Counties.

The Rural New Yorker variety was grown in most cases, although several other varieties were tested. Most of these plots were sprayed 3, and 5 or 6 times, and 5-5-50 Bordeaux mixture was used. Various methods of application were tried. Most of the potato fields were planted June 15 to 20, and the spray was applied at about two week intervals, beginning usually early in July. Of these 44 spray tests, increases in yield were obtained in 41 cases and in only 3 cases were slight decreases found on sprayed areas. The data obtained from the 44 plots may be summarized as follows: An average of 32.5 bu. per acre increase was obtained from all sprayed plots. This is an average of 29.5 per cent increase over the unsprayed plots. The average cost was \$10.27 per acre for spraying, and the average net profit from spraying during 1921 is figured at \$42.65 per acre. The more important results are summarized as follows:

No. of nozzles <sup>1</sup>	Pressure	Increase in yield per acre
2 (5 tests).....	75 lbs. (traction)	16 bu.
3 (16 tests).....	75 lbs. (traction)	27 "
3 (23 tests).....	200 lbs. (power)	39 "

*Relation of number of applications of spray to yield and net profit in Ohio*

No. sprays	Increase in yield per A.	Net profit per A. from spraying <sup>2</sup>
Traction sprayer		
3 (11 tests).....	18.7 bu.	\$22.42
5 or 6 (10 tests).....	36.4 "	\$36.80
Power sprayer		
3 (8 tests).....	30.7 bu.	\$42.21
5 (11 tests).....	44.4 "	\$61.30

The results from these spraying tests indicate a greater or lesser degree of control of hopperburn (tipburn), which was the major leaf disease in Ohio in 1921. Late blight was absent.

The most efficient spraying with Bordeaux 5-5-50, using 3 nozzles to the row, 200 lb. pressure and 5 applications, gave perfect control of tipburn from the point of view of the practical grower, and marked increase in yield.

Mr. Clayton also tested the complete potato dust as prepared by the Niagara Sprayer Co., and applied with a Niagara power potato duster. The results obtained on fields near Madison, Lake County, Ohio, are given in table 2.

<sup>1</sup> A number of men who sprayed with one nozzle to the row and 75 lbs. pressure reported no increase in yield.

<sup>2</sup> Computed from figures supplied by the growers.

TABLE 2  
*Results of dusting potatoes in Ohio in 1921.*

Coöperator*	Variety	Date planted	Dates dusted	Acres dusted	Yield per acre		Increase	
					Bu.	Bu.	Bu.	Per cent
Mr. Benjamin	Wellington	June 1	July 7, 21 Aug. 5, 19 Sep. 3, 7	$\frac{1}{2}$	111.6	106.2	+5.4	+5.1
Mr. Kelen	Wellington	June 10	July 21 Aug. 5 Sep. 3	$\frac{1}{2}$	49.2	40.4	+8.8	+21.8
Mr. Faust	Holloway	June 1	July 7, 21 Aug. 19	$\frac{1}{2}$	70.0	72.6	-2.6	-3.6
Mr. van Gulick	Unknown	June 1	July 7, 21 Aug. 5, 19 Sep. 3, 7	$\frac{1}{2}$	85.8	71.4	+14.4	+20.2

\* Three other coöperators reported no increase in yield from dusting but accurate yield data were not obtained.



*New Jersey.*—W. H. Martin obtained the following results in 1921:

1. Salem Co. A level field of Irish Cobblers on sassafras to sandy loam soil, fertilized with 2,000 lbs. 4-8-7 fertilizer per acre, was sprayed with home-made 5-5-50 Bordeaux mixture, except the check plots, which received only arsenate of lead. The Bordeaux was applied with a traction sprayer, three nozzles per row, 175 lbs. pressure, and 100 gallons per acre were applied. The first application of Bordeaux was made when the plants were seven inches high. No late blight, and only a trace of early blight, was present. Tip-burn was abundant. The sprayed plots remained green longer than the checks, the plots receiving 4 applications remaining green longer than those sprayed 3 times. Plots dug August 26, 1921; yield as follows:

Treatment	Yield per acre, bu.	Average
Check, (plots 1, 4, 7, 10).....	150.8, 155.7, 157.4, 129.5 resp.	148.3
Bordeaux, June 13, 20, July 1, 8, (plots 2, 5, 8).....	227.8, 239.3, 221.3 resp.	229.5
Bordeaux, June 13, 20, July 1, (plots 3, 6, 9).....	214.2, 213.5, 178.1 resp.	201.9

2. Cumberland Co. Irish Cobblers were planted on sassafras loam, and fertilized with 1800 lbs. 4-8-7 fertilizer per acre. The last four plots were on an area somewhat lower than the remainder of the field. Bordeaux was used as above, except that the dates were different. Early season sprayings were May 26, June 1 and 13. Late season applications June 20 and 29; all season spraying included all five dates mentioned. On June 29 flea beetles were numerous on the check plots (arsenate of lead only applied) and on plots receiving early applications of Bordeaux; very few on plots which had the late applications. No early blight by June 29, but some leaves yellow from dry weather. Plots dug August 10; yield:

Treatment	Yield per acre, bu.	Average
Check, (plots 1, 4, 7, 11, 14).....	90.2 109.6 128.0 131.6 134.5 resp.	118.8
All season, (plots 2, 6, 10).....	116.8, 127.9, 144.3 resp.	129.7
Early, (plots 3, 8, 12).....	120.8, 125.1, 152.8 resp.	132.9
Late, (plots 5, 9, 13).....	120.3, 136.1, 135.8 resp.	130.7

On the above field a test of 20-80 copper lime dust was made. The dust was applied May 26, June 13, 20, and 29 by means of a power duster. The dust did not afford much protection from flea-beetles and on June 29 little difference between check and dust plots could be noticed. The dusted area yielded at the rate of 120.7 bu. per acre, in contrast to 118.8 bu. on the check plot.

3. Salem Co. Irish Cobblers planted July 25 as a second crop for seed purposes on a level field of sassafras loam. The spray plots were sprayed with home-made 5-5-50 Bordeaux with a traction four-row sprayer with three nozzles per row. Dust plots received, in one series, 20-80, and in another 15-85 copper lime dust applied with a hand duster, and the plants were thoroughly covered. Check plots were not sprayed at all, since insects were not present. Dust and spray applied September 1, 10, 20, 27, October 5, 14. Plots dug October 29.

Results as follows:

Treatment	Per cent of leaves dead			Yield per acre bu.
	Oct. 5	Oct. 14	Oct. 21	
Check.....	61.2	91.1	98.0	125.0
Bordeaux.....	1.3	15.3	55.0	163.5
20-80 dust.....	14.0	43.3	88.0	144.1
15-85 dust.....	12.8	46.6	92.0	139.4

The check plots suffered considerably from early blight and tipburn. The dusted plots were affected to a lesser extent by these diseases, and the Bordeaux plots but little.

4. Salem Co. Late crop Cobblers. The following tests were made by growers who formed a "spray ring." A traction sprayer which developed 175-200 lbs. pressure was used, and 5-5-50 Bordeaux was applied at the rate of 100 gallons per acre, at a cost of \$2.06 per acre per application. Early blight and tipburn were the only diseases present. The yields in bu. per acre were as follows:

*Test I.* Sprayed 4 times, 160.1 bu.; check, 119.1 bu.

*Test II.* Sprayed 4 times, 213.5 bu.; sprayed 2 times, 198.6 bu. Check, 161.0 bu.

*Test III.* Sprayed 3 times, 213.4 bu.; check, 162.7 bu.

These results supplement previous data (see also N. J. Rept. 1920: 577-587, 1921 and N. J. Circ. 122) and indicate that spraying Cobblers in New Jersey will result in increased yield even in the absence of late blight. Fair results were obtained from copper-lime dust on the late crop, in spite of the dry weather, but during 1921 dusting did not give as good results as Bordeaux.

*Missouri.*—J. T. Rosa, Jr., reports tests upon Early Ohio potatoes from northern Michigan which were planted at Columbia, Mo., March 17, 1921, and given 4 applications of 4-4-50 Bordeaux during May and June, both with and without lead arsenate and nicotine sulfate. The sprayed plants remained green about 3 weeks longer, and when dug August 28 yielded 34.2 per cent more than the checks. However, the tubers from the sprayed plots bore so many knobby second growths that the actual quantity of marketable potatoes was less than that obtained in the check plots, which bore few secondary growths. The Early Ohio variety has been found previously to be more subject to second growths than other early varieties grown at Columbia. Perhaps other varieties, such as Irish Cobblers, would not react in this manner. The weather conditions during 1921 were probably conducive to secondary growth of the tubers, since short wet periods alternated with longer dry, hot ones. These conditions were particularly apparent the latter part of the season when only the sprayed plants were alive. Knobby tubers should not necessarily be less valuable than uniform tubers for seed purposes. Tests of soil moisture and temperature relations, and of the state of dormancy in tubers whose growth is checked by weather conditions, need to be made.

Leaf hoppers became abundant during the latter half of the growing season on the above plots. Very little early blight and no late blight occurred. Tipburn was serious.

Pyrox did not keep the plants green quite as long as Bordeaux, but the yield was increased just as much over unsprayed plots as was the case with Bordeaux.

*Kansas.*—E. A. Stokdyk, working in cooperation with L. E. Melchers, submits the following data obtained in cooperative potato spraying tests with M. R. Kelsey, Topeka, Kansas, in 1921. The plots were one acre in size. Home made Bordeaux mixture with poison was used. A power sprayer, equipped with two nozzles to the row and spraying ten rows at a time at a pressure of 150 to 175 lbs., was used. Approx-

mately 100 gallons were applied per acre. Check plots were sprayed with poison only. Plots sprayed 4 times had applications on May 23, June 3, 9, and 21. Plots sprayed 3 times had the May 23 spray omitted. Fields planted about March 30, and dug July 15 to August 15. Leaf hoppers present in the latter part of the season. Tip-burn and early blight about an average for Kansas, and as bad as or worse than in previous spraying tests. Late Blight does not occur in Kansas. The vines sprayed with Bordeaux remained green longer than the unsprayed ones.

TABLE 3

*Results of spraying Early Ohios in Kansas in 1921*

Spray applied	No. applications	Yields per acre, bu.	Cal. increase <sup>1</sup> per acre, bu.
Check.....	—	122.58	
Bor. 3-4-50.....	4	158.83	27.6
Check.....	—	139.88	
Bor. 3-4-50.....	3	191.80	43.7
Check.....	—	156.58	
Bor. 4-5-50.....	4	179.58	22.2
Check.....	—	158.25	
Bor. 4-5-50.....	3	160.00 <sup>2</sup>	5.9
Check.....	—	150.00 <sup>2</sup>	
Bor. 4-8-50.....	4	155.90 <sup>2</sup>	6.5 loss
Check.....	—	174.81 <sup>2</sup>	
Bor. 4-8-50.....	3	158.50 <sup>2</sup>	0.65
Check.....	—	140.90 <sup>1</sup>	

TABLE 4

*Results of spraying Irish Cobblers in Kansas in 1921*

Spray applied	No. applications	Yields per acre, bu.	Cal. increase per acre, bu.
Bor. 3-4-50.....	4	310.4	20.7
Check.....	—	289.7	
Bor. 3-4-50.....	3	310.9	24.6
Check.....	—	282.9	
Bor. 4-5-50.....	4	340.9	50.0
Check.....	—	299.0	
Bor. 4-5-50.....	3	279.1	5.4 loss
Check.....	—	270.0	
Bor. 4-8-50.....	4	285.5	34.2
Check.....	—	232.7	
Bor. 4-8-50.....	3	231.8	6.5
Check.....	—	218.0	

<sup>1</sup> Increase obtained by averaging the yield of two adjacent check rows, and subtracting the result from the yield of the sprayed row.

<sup>2</sup> Heavy rains had produced some irregular areas in these plots.

These results show an increase of 15.7 bu. per acre, or 10.5 per cent, from spraying Early Ohios, and an average of 21.8 bu. per acre, or 8.2 per cent, increase from spraying Irish Cobblers. Although these are not large increases, they indicate better results from Bordeaux under 1921 conditions than were obtained in the three previous seasons. The results up to date seem to indicate that with the methods used Bordeaux sprays for the potato crop in Kansas are of questionable value.

*Eastern Canada.*—P. A. Murphy gives (Canada Dept. Agr. Div. Bot. Bull. 44, 1921) valuable data on spraying in eastern Canada. The best results in fields were obtained from high pressure and three nozzles per row, although on small plots even better blight control can be obtained with hand sprayers. Bordeaux mixture was found to be better than Burgundy. Spraying before rains gave better results than spraying after rains. Many other data are given. The use of fungicides on potatoes in eastern Canada is rendered imperative by the prevalence of *Phytophthora*. An average of 130.5 bu. per acre yearly increase in yield of marketable potatoes was obtained from spraying with Bordeaux. Imperfect spraying, by keeping the vines green but not preventing development of *Phytophthora*, may result in more tuber rot than occurs on tubers from unsprayed fields.

G. E. Sanders finds that white arsenic may be combined with Bordeaux mixture and applied without injury to plants. (Agr. Gaz. Canada 7, No. 1, 1920; Proc. Ent. Soc. Nova Scotia 6; 8. 1921). He gives reports on dusting, but not specifically as applied to potatoes (Sci. Agr., Canada, Vol. 2, 1921; Ann. Rep. Fruit Growers' Assn. Nova Scotia, 1921: 66-92.)

*Pennsylvania.*—E. L. Nixon obtained in coöperative tests in 1921 even better results from the use of Bordeaux than those previously reported for Pennsylvania. Ten thousand one hundred and forty acres in 57 counties were included in spraying tests. An average increase of 74.3 bu. or 46.7 per cent was secured in the 402 demonstrations. Outfits yielding a minimum pressure of 200 lbs. were used in all cases. These results were obtained in a year with less late blight than occurred during 1918, 1919, or 1920. The spraying served to keep the plants alive over the extended drought which was fatal in many fields to the unsprayed plants.

*West Virginia.*—N. J. Giddings reports the following for 1921: At Wheeling, Irish Cobbler sprayed May 27, June 6, 20, and 30 with 5-5-50 Bordeaux mixture gave a 25 per cent increase in yield over the checks using hand sprayers. The plants were practically free from all leaf diseases except tipburn.

At Parkesburg, late potatoes (Carman) sprayed with hand outfit June 12, 16, and 27 gave a 20 per cent increase. Early potatoes sprayed May 21, June 2, 16, and 27 gave no increases. Tip burn was serious in both cases.

At Fairmount, early potatoes sprayed with hand outfits May 14, 26 and June 4, 17 and July 1 gave a 33.3 per cent increase.

At Davis three applications of spray using a power sprayer equipped with 3 nozzles to the row gave an average increase of 33.5 per cent in yield. A very little late blight was present at Davis.

*Kentucky.*—W. D. Valleau, Mr. Gardner and A. J. Olney report increases in yield and reduction in leaf hopper injury from the application of Bordeaux in 1921.

*Indiana.*—H. S. Jackson reports no authentic record of late blight in Indiana. Early blight is worst in the southern half of the state. Tipburn is very serious. Many commercial growers spray 3 to 5 times with 4-4-50 Bordeaux, which seems to be warranted by the partial control of tipburn secured.

*British Columbia.*—J. W. Eastham reports that spraying is not common over most of British Columbia because the Colorado beetle is absent, and late blight is absent over much of the province. Early blight may be destructive. An experiment at Sardis in 1918 indicated good results from Bordeaux against late blight.

*Arizona.*—J. G. Brown reports that Bordeaux checked the early blight attack in 1921 in Arizona. This disease spreads rapidly in Arizona during rainy periods unless the plants are sprayed.

H. W. Barre (*South Carolina*), J. G. Leach (*Minnesota*), J. A. Elliott (*Arkansas*) and M. B. McKay (*Oregon*) report no further data, nor have results for 1921 been received or noted from other regions not mentioned in this summary. Data from *North Dakota* by W. G. Colley and Dr. Weniger uncertain because of unfavorable weather.

#### SUMMARY

From the results obtained in 1921, it would seem that Ohio and Manitoba may be added as regions where the use of Bordeaux for potatoes is profitable. Supplementary data from New Jersey, West Virginia, Pennsylvania, certain other areas, and published results for Canada indicate further the value of spraying. Some further suggestions as to procedure, etc., may be gathered from the 1921 results. In the states of Kansas and Missouri, the value of Bordeaux is questionable.

During 1921 dusting trials on potatoes in New Jersey, Ohio and Manitoba gave results inferior to those obtained in spraying tests. This inferiority of dust in 1921 may perhaps be explained in part by the dry season and the absence of late blight in the areas where dust was tested.

## APPENDIX

### *REPORT OF THE FOURTH ANNUAL MEETING OF THE SOUTHERN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, AT LANTA, GEORGIA, FEB. 20-22, IN CONNECTION WITH THE GENERAL MEETING OF THE ASSOCIATION OF SOUTHERN AGRICULTURAL WORKERS.*

The meeting of the Southern Section of the American Phytopathological Society held at Atlanta, Georgia, in conjunction with the general meeting of the Southern Agricultural Workers was poorly attended, although fairly well represented by workers from the Southern States which are near to Georgia. It is unfortunate that such meetings are not better attended because problems of immediate concern are usually taken up which should interest southern plant pathologists. The meager attendance was probably due to the fact that many of the workers attended the general meeting at Toronto and felt that they could not attend both.

The program was arranged in a manner more or less similar to that carried out last year, that is, part of the time was given to a joint session with the Horticultural Society and the other part to the meeting of phytopathologists alone. Considerable prominence was given to a discussion of the sweet potato and its diseases which was really in the nature of a symposium and which consisted in reviewing work previously done. The object of this symposium was primarily to interest the horticulturists and have them present their side which deals with the cultural end of this important southern crop. As a criticism, it might be said that few original papers were presented which might, perhaps, indicate a lack of interest. In the future, it is planned to make these meetings more interesting and have more papers presented on some definite problem of plant pathology which concerns the Southern States.

The following officers were elected for the ensuing year:

Chairman—Professor H. W. Barre, Director of South Carolina Experiment Station.

Vice Chairman—Professor S. H. Essery, Mycologist and Plant Breeder, University of Tennessee, Knoxville, Tennessee.

Secretary—Dr. J. J. Taubenhaus, Chief, Division of Plant Pathology and Physiology, Texas Agricultural Experiment Station, College Station, Texas.

Representative to the Council of American Phytopathology—Dr. L. R. Hesler, Botanist and Plant Pathologist, University of Tennessee, Knoxville, Tennessee.

Upon motion it was agreed that the Association of Southern Agricultural Workers be asked to appoint a committee to work on a cooperative project on root knot, this being an extremely serious disease in all the Southern States, the committee to consist of a plant pathologist, an entomologist, a horticulturist, and an agronomist. In the absence of Dr. Eliot, Professor H. W. Barre acted as chairman.

Respectfully submitted,

J. J. TAUBENHAUS, Secretary.

## ABSTRACTS OF PAPERS READ

*Recent studies of Texas root rot of cotton.* J. J. TAUBENHAUS.

Studies were reported on the life history of *Ozonium omnivorum* as it affects the cotton. It was found that the fungus does not live over in the soil, nor on decaying organic matter, but that it requires a living root on which to pass the winter. Roots of cotton plants, wild morning glory, castor bean, okra, pepper, and probably many others afford a root host on which the *Ozonium* winters over. Successful inoculations were also reported for the first time, using *Ozonium omnivorum* and successfully infecting healthy cotton plants. The method employed was somewhat different from the usual way. It was also reported that spores corresponding to the *Phymatotrichum* stage which Duggar described some eight years ago were found on pure cultures, using sterilized soil as a medium. Extended studies covering a period of seven years were made in which control methods are practically developed. The results of this work will be published in a station bulletin shortly.

*The Ascochyta blight of cotton.* JOHN A. ELLIOTT.

The *Ascochyta* blight of cotton, attributed to *A. gossypii* Sydow, was locally severe in west central Arkansas in June, 1920. Investigation showed that a serious epidemic of the same disease had occurred in the same region in August, 1915. Both outbreaks took place during periods of excessive and continued rainfall. A less serious outbreak occurred in June and July of 1921, under similar conditions. From circumstantial evidence, it is thought that the disease is at present of local occurrence. Potentially, it is a disease of great importance, a period of continued wet weather being the only condition required for the complete destruction of the cotton crop when the disease is present. Dry weather checks the advance of the disease.

*Corn mosaic in Arkansas.* H. R. ROSEN.

Corn mosaic is reported as having been collected in Mississippi County, Arkansas, in 1921.

*The control of Root-Knot. A progress report.* J. A. McCLINTOCK.

Experiments conducted by the writer at the Georgia Experiment Station during the past three years have shown the possibilities of root-knot nematode control through the use of resistant plants. Various plants have proven resistant to the root-knot nematode, the most recent addition to the list being a seedling peach. Seed of this peach have been planted to determine whether the factor of resistance is seed transmitted in the case of this peach. As data are collected a report will be made relative to this point, and in the meantime workers should watch for resistant individuals among various plants which are infested by root-knot nematodes.

*Dusting vs. spraying for the control of the curculio, brown rot and scab of peaches.* OLIVER I. SNAPP.

Data from two years' work in Mississippi and one year's work in Georgia show the liquid spray to be somewhat superior to the dust for the control of the curculio under heavy infestations, but for brown rot and scab control the two methods of pest control gave about equal results. The 80-5-15 dust formula gave practically as effective control of curculio, brown rot and scab yearly as the 80-10-10 or other dust formulas

containing more arsenate of lead. An application of arsenate of lead when seventy-five per cent of the petals were off and another four weeks before the fruit is due to ripen in addition to the treatments when the calyces were shedding and again in two weeks is strongly advised, especially in latitudes where two generations of the curculio occur.

*Field and storage diseases of the sweet potato and their control.* L. L. HARTER.

The diseases of the sweet potato may be roughly divided into three groups, as follows: (1) diseases of the leaves, (2) diseases of the root and stem, and (3) storage rots.

The diseases of the first group require no remedial measures. To control diseases of the second group care should be given to seed selection, seed disinfection, care in the preparation of the hot bed, and crop rotation. The seed potatoes should be selected in the fall at digging time and again in the spring just before bedding. A new hot bed should be made each year. The seed potatoes should be disinfected for from 5 to 10 minutes in a solution of mercuric chloride made by dissolving 1 ounce of mercuric chloride in 8 gallons of water.

To keep potatoes in storage they should be dug just preceding frost and handled carefully. The storage house should be kept dry and at a temperature of about 50 to 55° F.

*Notes on the physiology of the sweet potato.* WRIGHT A. GARDNER.

This paper consisted of a review of the literature of work on sweet potatoes, with special reference to transformations of carbohydrates.

Results of preliminary investigations were reported which indicate that there is little or no injury when tops of sweet potato plants are removed two weeks before digging; that topping and severing of roots results in considerable rotting; that cured sweet potatoes are not injured by the outdoor conditions which prevail when the temperature falls to 3.8° C during a frosty night; and that the expressed juice of sweet potatoes shows a depression of freezing point amounting to 1.28° C.

*Fusaria of corn.* C. D. SHERBAKOFF.

The author's recent studies of *Fusaria* associated with corn seed show that there are several different species of this genus more or less commonly isolated from the seed, some of the species resembling each other in their appearance on agars ordinarily used in a similar work. It is suggested that there might be a difference also in the pathogenicity of these *Fusaria*. Because of the great importance of corn rootrot in Tennessee, the local experiment station is starting this season certain experiments with corn to determine (1) the different *Fusaria* associated with corn seed, (2) the correlation between the results obtained from rag-doll and sand-box tests of the seed and rootrot in the field and (3) the effect of certain methods of seed treatment on corn rootrot. Other plant pathologists of the South are invited to cooperate in the work.

*The less common spray materials.* N. D. PEACOCK.

All new spray materials should prove themselves better than the standard materials before they will be accepted for general use. They should be compared with the standard materials in efficiency, cost, convenience and danger of injury.

Practically all of the proprietary fungicides have either copper or sulphur as a basis and are therefore directly comparable to either Bordeaux mixture or lime-sulphur.



Some of the newer much advertised spray materials are very promising and deserve consideration, others are of indifferent value and some perhaps are decidedly objectionable. Growers should use all of these materials experimentally (if at all) before adopting them for general use.

*Three years sweet potato certification work in Arkansas.* GEO. G. BECKER.

The sweet potato certification service which is being conducted by the Arkansas State Plant Board has been in operation for three years without modification and is doing every thing that was expected of it. Through sheer merit and consistent dependability Arkansas certified seed and slips will in time make it difficult to dispose of sweet potato seed and slips on any large commercial scale unless they are certified.

In addition to the present requirements for getting a sweet potato slip certificate we will this year require that the location of the slip bed be approved by an inspector of the board before seed can be bedded down for certified slips. Experience teaches us that purchasers of certified seed must be made to understand that certified seed is not immune to diseases. We accordingly supply all of our certificate holders with information to give to their customers and we find that they are glad to distribute this for their own protection. We are thoroughly convinced that no certification service can be conducted unless we have in our employ, or can work in close coöperation with a thoroughly competent Plant Pathologist.

*"Mosaic" disease of corn in Arkansas.* H. R. ROSEN.

"Mosaic" disease of corn has been noted for two successive years in northeast Arkansas. Comparatively few plants were found attacked and of these only a small number were so seriously affected as to influence the production of normal ears. Badly diseased plants are very much stunted, due principally to a shortening of upper internodes, possess chlorotic spots and stripes in great profusion, and contain a compact group of shortened leaf-blades, presenting a rosette appearance, at the top of the plant. Such plants produce abortive ears only. Present observations indicate that under Arkansas conditions the disease is not as serious or as common as in the Hawaiian Islands.

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## MOSAIC AND LEAF CURL (YELLOW) OF THE CULTIVATED RED RASPBERRY

W. H. RANKIN AND J. F. HOCKEY

After two years spent in a preliminary investigation (mostly observational by force of circumstances) of the disease which has been known as raspberry yellows, it is believed that a progress report is warranted. The previous literature on yellows is meager and confined chiefly to brief descriptions of the diseased plant, the suspected causes of the disease and suggested control measures. The possible causes suspicioned have included insects, fungi, bacteria, excessive soil water and unbalanced nutrition. Green (6) in 1895 states that for 5 years a disease known as leaf curl has seriously affected almost every variety of red raspberry on the grounds of the University Farm (Minnesota), and that its ravages were very wide spread in that district. Stewart (11) in 1902 described a serious disease of the variety Marlboro in New York State which he called yellows, because the reaction of the plant suggested peach yellows. Clinton (2) in 1903 briefly states that yellows as described by Stewart is present in Connecticut and is more severe on soils poor in nitrogen. Melchers (7) in 1914 described leaf curl which he believed to be the same as yellows. He gives many facts derived from observations in Ohio regarding its importance and possible cause. He also attempts to separate yellows or leaf curl as a disease distinct from other raspberry diseases which are characterized by a "yellowing" or sickly foliage. Clinton (3) in 1915 included yellows of raspberries in his discussion of mosaic diseases and briefly described the affected plants. Weiss<sup>1</sup> in 1918 described leaf curl from observations made in Minnesota and Manitoba. He also attempted various methods of inoculating healthy plants in the green house and in the field, and studied the effect of transplanting to different types of soil and the influence of different amounts of soil water. He did not succeed in transmitting

<sup>1</sup> Weiss, Freeman. A manuscript report on work done at the University of Minnesota, a copy of which was kindly furnished to the writers by Mr. Weiss.

the disease or in finding any connection between the type of soil and the amount of soil water in causing or relieving the symptoms of leaf curl. Dickson (5) in 1921 states that it is possible that mosaic and curl or yellows have been confused and that it remains to be seen whether or not curl is an advanced stage of raspberry mosaic. Several compiled bulletins and circulars describe yellows or leaf curl (used synonymously) giving the symptoms of both the mosaic and leaf curl as characteristic of the disease.

The above brief review of the literature on yellows or leaf curl is sufficient to call attention to two facts; first, that practically nothing is known except the symptoms and second, that there probably are two distinct diseases, one a mosaic and the other a leaf curl. As a result of the facts now available and reported below it may be stated that it is practically certain that Stewart described the mosaic and called it yellows, and that Melchers and Weiss were working with leaf curl alone, but confused the symptoms of leaf curl with the published descriptions of yellows and used the names as synonyms. The two diseases mosaic and leaf curl are distinct. Since the name "mosaic" has been very generally adopted for a type of disease with which Stewart's "yellows" conforms in all important respects and especially on account of the confusion which has occurred it might be better to abandon entirely the name "yellows."

Like other mosaic and leaf rolling or curling diseases these two raspberry diseases are difficult to describe fully in all their symptoms. The initial symptoms of the mosaic are more easily recognizable in some varieties than in others, and, under certain weather conditions the characteristic foliage mottling is more or less obscured. In these respects raspberry mosaic is subject to many of the uncertainties as regards diagnosis that are found in potato mosaic. On the other hand raspberry leaf curl, which has been found to be similar in many respects to potato leaf roll, has such well defined symptoms that one rarely need be uncertain of its diagnosis.

The work reported below was confined largely to the intensive fruit district of the Niagara Peninsula in Ontario. This district extends along the south shore of Lake Ontario from the Niagara River to the western end of the lake and for several miles along the northwestern shore. The red raspberry (*Rubus strigosus* Michx.) is one of the principal small fruits grown and the plantings aggregate several hundred acres. At least 90 per cent of this acreage is planted to the variety Cuthbert. A few plantings of Marlboro and Herbert make up the remainder.

Three brief accounts (8, 9, 10) have been published and a report (Division of Botany, Annual Report 1921-1922) in more detail is in press; all of which are based on the same work that is summarized below.

#### SYMPTOMS OF LEAF CURL

Leaf curl is a systemic disease, in that all of the new growth, formed subsequent to infection, is more or less typically affected. The leaflets on the first- and second-year wood are much darker green than normal and the mid-vein arches downward throughout its length. A similar arching of the lateral veins causes a downward curling of the entire margin of the leaflet. Very often a greater tension in the arching of the mid-vein results in the tip rolling under. Another constant characteristic of the curled leaflets is the "gathering" of the interveinal tissue along the mid-vein and lateral veins. Also the interveinal tissue is often "puckered" and arched between the lateral veins. These results of uneven development are more pronounced when the plants are growing rapidly and the tissues are succulent. When growth is less rapid or the infection of the plant has been recent, the "gathering" along the veins in certain areas may be marked, without much if any resultant curling. Not all cases of slight "gathering or puckering" along the veins, however, are attributable to the leaf curl disease. Very slow growth in the early spring, insect injuries and continued hot dry weather often cause the same effect. In such cases, as soon as these conditions are passed the new growth is normal. In the case of leaf curl all subsequent growth without regard to weather conditions is typically curled. The deformation usually becomes more pronounced as the season advances, until in the case of first-year canes, the tip is stunted and ends in a few small light yellowish green and severely curled leaves. The symptoms of curl are similar for the three varieties, Cuthbert, Marlboro and Herbert. In fact, the leaf characters of these varieties are so masked in the curled plant that it would be difficult to distinguish the variety.

Usually the first-year wood, if infected early in the season does not reach the normal height. If infected in midseason or later, the effect of the disease which results in the dwarfed tip is not reached. In this way curled first-year wood may or may not be dwarfed depending upon the time of infection and the vigor of the plant. The fruiting laterals on a diseased second-year cane are short, upright in habit and the leaves are small, dark green and more or less typically curled. Frequently

the tissue midway between the lateral veins loses its green color and becomes blanched or slightly bronzed. This type of mottling is easily distinguished from that which is characteristic of the mosaic disease. The fruit which develops is small, has very little pulp and is lacking in flavor. In all respects, the effect on the fruit is similar to that produced by mosaic and anthracnose.

Curled leaves are developed in 4 to 6 weeks after infection. All branches or new suckers developed from the stem or roots after curled leaves are formed at the tip, are typically curled. All the leaves developed previous to infection remain normal. The new canes formed the year after infection are much shorter than normal. Individual plants have not been observed for sufficient length of time to enable one to draw definite conclusions, but it is believed that after 3 or 4 years the canes develop only to a height of a few inches and that in this way the disease is finally fatal.

Leaf curl in many respects suggest similarity to potato leaf roll in its effect on the plant. The fundamental foliage symptoms are the same, suggesting inhibition of the translocation of starch, or some disturbance which results in starch accumulation. The stunting habit and the general dwarfing effect is common to both diseases. Also both are systemic and the histological studies have shown a necrosis of certain elements of the phloem and pericycle in the raspberry which resembles potato phloem necrosis. Also both are aphid disseminated. Although the two diseases may be alike in gross symptoms, without there being much in common in regard to their cause, it is at least plausible to suspect that raspberry leaf curl and potato leaf roll both belong to the same group of related diseases and that their causes may be similar in nature.

#### SYMPTOMS OF MOSAIC

Mosaic is noticeable in a plantation from a distance because of the dwarfing of the canes, the sparse yellowish foliage and thin growth. Once mosaic appears in a row of plants it soon spreads, so that for the distance of several meters every cane is affected. The fruiting canes near the middle of the long diseased areas (diseased for more than 3 years) are short and very slender. They may be only little more than half as tall as healthy canes and often less than half the diameter. The laterals developed on these dwarfed canes approach the average length of healthy laterals, but they are spindly. The leaves are not over one-half the size of those on healthy plants. Many of the leaves show the

large, green, blister-like mottling while others show only slight mottling. The leaves have a dull green or even yellowish appearance in contrast with the shiny green of the healthy leaves. The veins are more prominent than normal and appear to be slightly sunken, causing a fine marking of the upper surface. These leaves soon bronze or turn yellow-green in the summer. The suckers from the roots of such canes are usually shorter than the canes of the preceding year and show distinct coarse and fine mottling in all the leaves.

The fruit developed on a cane which shows the dwarfing effect is largely worthless. Much of the fruit on mosaic canes did not develop a pulp during 1921 which was hot and dry at the time the fruit was maturing. That which did develop a pulp was tasteless. Where there was a large percentage of mosaic, the quality of the fruit was greatly impaired even though the dry and seedy berries were not picked.

All the symptoms above described for a plant which has been diseased for probably three years or more are also shown, but often to a less degree, in plants which have been affected only one or two years. Fruiting canes and suckers near the ends of diseased areas are almost as tall and as large in diameter as healthy canes. The leaf symptoms on the fruiting canes are the same as described above except that there is probably less tendency to bronze and turn yellow. In some cases, tall sturdy fruiting canes normal to all appearance, except for the definite leaf mottling, have been noted with mottled suckers which are also normal in height and diameter.

At the ends of the diseased areas there are often found two stages of the invasion into the healthy plants. There are some plants, with one or more of the suckers showing marked mosaic symptoms from the oldest leaf to the tip, and others in which the suckers show no signs of mosaic except in the tip leaves and these may be either finely or coarsely mottled. These two conditions are due to whether infection occurred early in the spring or during the summer.

The most constant and diagnostic symptoms of this disease are shown in the leaves on the suckers. No difference has been noted between those coming from roots diseased for several years and those apparently infected in the spring when they started growth. Before the middle of June the leaves show large irregular green blisters which arch upward. The blisters are not confined to the arrangement of the veins in any way. The tissue between these blisters is lighter green than normal or has a yellowish appearance. Severely blistered leaflets curl downward while the mid-vein remains straight and thus forms a longitudinally rolled

leaflet. In 1921 very high temperature and drought conditions prevailed for about 6 weeks beginning the middle of June. During this time the suckers grew slowly and made short internodes. The leaves were normal except that the leaflets were broader and the tip curled under. A dry corky area was always to be found on the under surface of the mid-vein about one-third of the way from the base of the leaflet. The leaves formed during this period on affected suckers did not show any mottling. During August and September under normal conditions of moisture the suckers made the usual growth with normal foliage. All suckers, which had shown the large blister-like mottling in the spring now developed a fine yellowish speckled mottling, without any rolling of the leaflets. Also recently infected suckers, which showed no mottling in the vernal foliage, developed the fine mottling. In some cases coarse blister-like mottling was produced on some leaves, but this was exceptional.

Many of the Cuthbert suckers affected by mosaic (probably one-fourth) form 1 to 6 or more laterals from the axils of the leaves. These grow into long leafy branches and the axillary buds on them develop fruiting laterals the next year. Such suckers do not grow to normal height.

There is some difference in the appearance of mosaic on the three varieties grown commercially in the Niagara district. In the Cuthbert with its flat, thin, light-green leaf, the mosaic mottling shows very prominently. In the Marlboro with the leaves more or less rolled and fluted due to an arching of the tissue between the lateral veins, the mottling is not distinct, except when abundant and where the yellowish areas are large. The natural dark green of the leaf also seems to mask the finer mottling more than in the Cuthbert. In the Herbert, with leaves similar to the Marlboro as regards color and fluting, the mottling seems to show more prominently than in either of the other two varieties. Even slight mottling results in distinct yellow areas in the dark green leaf and pronounced mottling results in a yellow leaf. The character of dwarfing is shown by all three varieties. More Cuthbert suckers showing pronounced mottling are found which are not markedly dwarfed than in the case of the Marlboro and Herbert. In these two varieties the canes are dwarfed severely and uniformly. The difference in dwarfing is apparently due to a time factor. It is believed that the Marlboro and Herbert respond very quickly after infection and are soon dwarfed. The Cuthbert seems to be slower to respond and usually is not markedly dwarfed until 2 years after infection.

Cases of mixed symptoms of leaf curl and mosaic have been found in the same plant or in the same diseased area in only two instances. The evidence was clear that the plants were affected by both diseases and different parts of the same leaflet often showed the characteristic symptoms of each disease. It is believed that it may not be uncommon to find the two diseases occurring simultaneously. The previous confusion of the symptoms of leaf curl and mosaic in other districts may be due to a high percentage of mixed infection.

#### ECONOMIC IMPORTANCE

There are too few published statements on which to base estimates of the losses due to leaf curl and mosaic. One or the other or both diseases are probably present in all of the larger small-fruit districts of northern United States and Canada. Melchers (7) and Weiss<sup>1</sup> found leaf curl in Ohio, Michigan, California, Washington, Minnesota and Manitoba. To this range may be added all parts of Ontario and Quebec, Prince Edward Island and Nova Scotia. Leaf curl is also a disease of some importance in Illinois (1) and New Jersey (4). So far as mosaic is concerned there is no definite information concerning its range. It is known to the writers to be present in Nova Scotia, Prince Edward Island, Quebec, Ontario, Michigan, New York and Connecticut. Within this wide range, leaf curl and mosaic have been credited with serious destruction. The "running out" of varieties, especially the Marlboro and Cuthbert and the marked decline in acreage in many districts have been credited to one or the other of these two diseases.

A survey of the conditions in the Niagara Peninsula of Ontario was conducted during the spring of 1921. In this district, which is about 60 miles long, counts were made for the amount of leaf curl in 136 commercial plantings of the variety Cuthbert. While over 10 per cent leaf curl was found in many plantings, the highest average for the different local districts was 6 per cent and the general average was about 4 per cent. In 18 plantings of Marlboro and 27 of Herbert, there was an average of less than 1 per cent leaf curl. The average amount of mosaic in plantings of the variety Cuthbert was about 20 per cent. Plantings of Marlboro showed an average of 27 per cent mosaic, while in the variety Herbert there was only about 1 per cent mosaic.

In the district under discussion leaf curl is enphytotic and although the average loss in stand of 3.8 per cent for 1921 is not large in itself, the cumulative loss over a period of years is an important factor. Most

<sup>1</sup> Weiss, Freeman. Manuscript previously cited.



growers rogue the plants affected by leaf curl in June to prevent the undesirable fruit from being picked. The spaces are usually left unfilled. Roguing in June has had very little if any effect in the control of leaf curl and therefore the percentage of missing plants has increased from year to year. Under the present methods of culture in the district, it is estimated that on the average, a grower has been losing a total of about one year's crop in the life of a plantation or about 8 per cent in gross returns annually.

The losses from mosaic are more difficult to determine, Mosaic has become epiphytotic in the last two years and if it continues, the present indications are that the majority of the plantings of Cuthbert and Marlboro will be destroyed. The practice has been to obtain stock for planting from a neighbor's fruiting plantation. In this way many plantings have been set in the last few years with a high percentage of mosaic plants scattered through them. Plantings made only 3 or 4 years ago have been found with over 30 per cent mosaic as contrasted with plantings at least 10 years old with less than 5 per cent mosaic. It is not uncommon, however, to find in plantings both young and old, numerous areas of mosaic in the rows which are from 3 to 7 meters long. It is evident from the dwarfing that these mosaic areas are often the result of spread in both directions in the row from a single infected plant. The grower has not been accustomed to rogue the plants affected by mosaic and therefore the amount of the disease has increased yearly. The yield of Cuthbert plants affected for 2 years or less is usually not seriously reduced. After 2 years the small number of suckers developed and their dwarfed nature, together with the tendency of the fruit that is set to have very little pulp, leads to a great reduction in yield and to very poor quality. Mosaic is estimated to have reduced the yield from the affected plants in 1921 about 50 per cent. With this estimate as a basis the average loss in gross returns per acre for Cuthberts was about 10 per cent.

#### SPREAD OF LEAF CURL AND MOSAIC

Both diseases in this district spread only slowly from a diseased plant to neighboring plants. The original roots in continuous rows of plants often are obscure but in general it is true that leaf curl spreads only to the next adjacent plant in a year's time or at most to the next two. Mosaic appears to spread a little faster and an annual spread to the next two plants is common. Newly infected plants appear annually at some distance from old infections. These new areas of infection rarely amount

to more than one-fourth of the number of previous areas. It is thus seen that if the original percentage of either disease is low at time of planting that it takes several years to account for high percentages. The plant affected by mosaic, especially in its earlier stages, is often not recognized by the grower as a diseased plant. It is believed from all the field evidence that the present difference between the enphytotic nature of leaf curl and the epiphytotic nature of mosaic in this district is due largely to the amount of each which has been carried over into the new plantings.

The only plausible and constantly associated agent of inoculation that has been found so far is the aphid, *Aphis rubiphila* Patch. This aphid while never occurring abundantly in this district is commonly found in small scattered colonies. From the writers' observations the following tentative facts have been ascertained regarding the life history of this aphid. The eggs are laid in the fall on the cane in the crevice between it and the buds. The stem-mothers emerge sometime in May, shortly after the fruiting spurs begin their development. The first abundant colonies of young are found on the new suckers late in May and in June, and there is a rapid dispersal at this season. During the summer months, according to weather conditions, the aphids either practically disappear or exist in small colonies in a more or less stationary position. There is practically no movement on the plant or to other plants during the warm weather. In the autumn, cooler weather and frequent rains lead to another season of rapid multiplication and dispersal. So far as known, no winged stage is developed and there is no migration to other food plants. Counts made in the early spring have shown that the sucker affected by mosaic due probably to its more succulent foliage is a more favorable food plant for the development of colonies than is the healthy sucker. The sucker affected by leaf curl is also more favored by the aphids than is the healthy sucker but to a lesser degree than that affected with mosaic. In this way the character of the newly affected suckers enhances the chances of dissemination by harboring larger and more active colonies of aphids than do the healthy plants.

Many different ways of artificially inoculating several hundred healthy plants with the causative agents of leaf curl and mosaic have all failed. Provision for developing selected strains of aphids were made in 1921 but the work of transferring the aphids to healthy plants was only begun just previous to the severe drought which practically eliminated the aphids and made further work impossible. In six instances leaf curl

was successfully transmitted by transferring aphids under conditions where other factors were eliminated. The first set of transfers for mosaic transmission, however, were made at the beginning of continued hot weather and no results were obtained. The field evidence is so conclusive that there is little doubt that both diseases are transmitted by *Aphis rubiphila*.<sup>1</sup>

#### VARIETAL SUSCEPTIBILITY

Plants of the following red and purple varieties have been seen affected by mosaic: Abundance, Brighton, Brilliant, Columbian, Count, Cuthbert, Dr. Reider, Eaton, Empire, Golden Queen, Haymaker, Herbert, Highland Hardy, Idaho, June, King, Louboro, Marlative, Marlboro, Marldon, Minnesota, Newman No. 1, Newman No. 23, Newman No. 24, Ontario, Royal Purple, Ruby, Segrist, St. Regis. The data were obtained largely from an examination of the horticultural plots of the Central Experimental Farm, Ottawa, Canada, and of the New York Agricultural Experiment Station, Geneva, New York. A mosaic, similar in all gross symptoms at least, is common on some cultivated varieties of black raspberries.

According to Weiss<sup>2</sup> leaf curl is a common disease of the wild *Rubus strigosus* Michx. in Minnesota and in Manitoba as far north as the upper end of Lake Winnipeg. It has also been found common on the same host in northern Ontario. The variety Cuthbert is very susceptible but the Marlboro and Herbert are rarely affected. In one instance an unknown variety of black raspberry was found with typical leaf curl. Also 3 of 5 ornamental wineberry bushes (*Rubus phoenicolasius* Maxim.) were found affected by leaf curl.

#### CONTROL MEASURES

The first and most important factor in the control of either leaf curl or mosaic would seem to be an attempt to produce healthy planting stock for local use. Present indications are that stock in small isolated plots can be kept healthy for transplanting without much difficulty. The most of the increase in the fruiting plantation is from plant to plant. New areas are most easily accounted for by the dragging of diseased canes during the cultivating and pruning operations, or by the occasional

<sup>1</sup> Prof. B. T. Dickson, of Macdonald College (Quebec) who has been working on several mosaic diseases, reported verbally during the discussion on raspberry mosaic at the Toronto meetings that he had successfully transmitted this disease in three instances by transferring *Aphis rubiphila* to healthy plants.

<sup>2</sup> Weiss, Freeman. Manuscript previously cited.

transfer of the aphids themselves by strong winds. It is not believed that a plantation set with healthy stock will be subjected to much chance of infection, if it is separated from other plantings by a distance of as much as 30 meters. These statements are borne out by general observation where high percentages are clearly due to a large number of old diseased areas and by many old plantings which show very few such areas. In the case that in some districts the disseminating agent develops a winged stage or has other hosts, the above arguments would have but little weight.

In young plantings where the amount of either disease is not too great, control may be possible by roguing at a season when the aphids are more or less stationary. In this district leaf curl is easily distinguishable shortly after the growth begins in the spring and before the aphid eggs hatch. Present experimental control measures for leaf curl are based on removing the affected plants during this interval. The symptoms of mosaic are not distinguishable in the spring until after the aphids are dispersed in large numbers. It is believed, however, that normally the aphids are stationary enough in mid-summer to insure that all affected plants have had a chance to develop symptoms since infection. Experimental control for mosaic by roguing has been limited to an indefinite period, beginning about two weeks after continuously warm summer weather has begun. It is true that in excessively hot weather the recently infected plants do not show the symptoms of mosaic, a condition which interferes with successful roguing.

No attempts apparently have been made to develop a desirable commercial variety either by selection or by breeding which is resistant to either of these diseases. For diseases of this type it would be fortunate if resistant varieties could be produced since the propagation of disease free stock and constant roguing in the hands of the grower are very unsatisfactory measures to depend upon.

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# FASCIATION AND PROLEPSIS DUE TO CROWN GALL

ERWIN F. SMITH

WITH PLATES XVI TO XX

Several times in recent years (*Science*, n. s., 1916, Vol. 44, p. 781; *Jour. of Agric. Res.*, 1916, Vol. 6, Pl. XVIII; *Introduction to Bacterial Diseases of Plants*, 1920, text and figs. 335, 340) the writer has called attention to the fact that fasciation of shoots may be one of the results of crown-gall inoculations, but as these remarks were incidental in writings devoted to various phases of crown-gall research not much attention appears to have been paid to them. I propose, therefore, to call attention to the subject once more directly and to show an additional example.

The fusion and flattening of various organs known as fasciation is very common in a great variety of plants and, being often so striking as to cause great wonder, many good pictures have been published in books and papers on plant Teratology. A similar phenomenon occurs in the animal world and there are many interesting figures of trunk and limb duplications or fusions, especially of parts of the human body, in treatises on "Monsters." Recently Stockard and others have attacked the problem experimentally.

There have been many speculations among botanists as to the cause of these abnormalities, but thus far very few enlightening comments.

The cause of fasciation is a problem to be approached and solved by experimental methods, rather than by observation, and the following remarks may be considered in the light of suggestions as to the direction of approach in individual cases rather than as a key to the whole situation.

I have demonstrated that fasciation can be produced experimentally by inoculating *Bacterium tumefaciens* Sm. and T. into the leaf axils of a variety of plants (*Nicotiana*, *Pelargonium*, *Ricinus*, *Brassica*, *Tropaeolum*) and the immediate presumption is that many other fasciations are disturbances due to the penetration of foreign organisms into the growing point in such a way as to crowd and divide it or to bring about irritation leading to fusions.

Each particular fasciation is a problem by itself and I would not be understood as contending either that all fasciations are due to one and the same microorganism or that all are due to parasites and their products; but only that the hypothesis of a symbiont or of a feeble parasite

(bacterial, fungous or other) offers a hopeful method of attack in individual cases.

In any event, the initial disturbance may be assumed to have begun in the embryonic or dormant bud stage, and in attempting to culture out an assumed parasite one should begin, I think, at the base of the fasciation as the part most likely to contain it. This conclusion is based, in part, on our work on hairy-root in which it was shown (U. S. Department Agriculture, 1911, Bureau of Plant Industry Bulletin 213, p. 101) that the organism occurs in the flat base from which the tuft of roots arises but not in the roots themselves; in part, on recent observations that the crown-gall tissue occupies only the base in such shoots as are described in this note. This absence of the causal organism in the greater part of the fasciation would also serve to explain why examinations of fasciated shoots have always proved sterile.

The first three figures shown (Pls. XVI, A, B, and XVII) are of a nasturtium plant (*Tropaeolum majus* L.) inoculated when young in a leaf axil with *Bacterium tumefaciens* Sm. and T. by means of needle pricks and photographed a month later when the only secondary shoot on the plant was the fasciated one from the inoculated axil. At the time the inoculation was made there was, of course, no shoot in this axil nor even any visible bud. The fasciated shoot developed from a dormant bud along with the growth of the tumor and in consequence of its growth. In other words, the development of the shoot as related to the tumor is not merely a *post hoc* phenomenon, but one actually due to the presence of the tumor. Plate XVI A shows the whole plant about  $\frac{1}{3}$  natural size with the fasciated shoot at its base and all other leaf axils free from shoots. Plate XVI B and plate XVII show respectively the lower and upper one-half of the fasciated shoot, enlarged  $\times 5$  for the sake of details. In six leaf axils on this fasciated shoot there was an abnormal pushing of buds just as I showed recently for tumor-stimulated shoots on *Bryophyllum calycinum* (Effect of Crown Gall Inoculations on *Bryophyllum*. *Jour. of Agric. Res.*, Vol. XXI, July 15, 1921, plates 101 and 107).

At the time this fasciated shoot was photographed and fixed for sections there was no evidence of tumor tissue anywhere on its surface, but after fixing in Carnoy's fluid ( $\frac{1}{4}$  glacial acetic acid,  $\frac{3}{4}$  absolute alcohol) the flattened shoot became very clear except for internal yellowish areas in the lower one centimeter which were assumed to be due to crown-gall proliferations in the vascular region and shown to be such in stained sections. This proliferating (invasive) tissue is present in.

ternally at the base of the shoot, as far up as the arrow, but not in the tissues midway or farther up the shoot. The effect, however, of tumor proximity was a general stimulus of the whole shoot including its dormant axillary buds. The primary tumor is visible in plate XVI *B* at *T*, *T*, with the shriveled base of the subtending petiole at *P*, and below this in the main axis the tumor has extended downward a short distance and burst through the cortex as indicated at *S*. There are also clusters of incipient roots (*R*). In the upper part, also, (Pl. XVII) axillary buds are pushing at *X*, *X*, *X* and from the axil of *L*. At *A* and *B* there are two distinct axes of growth. There is a third axis of growth (fasciation) on plate XVI *B* at *F*. The contrast between the size of the main axis *M* and of the fasciated shoot indicates clearly a strong movement of water and elaborated foods into the tumor and the shoot complex.

The plant was inoculated when it was a month old and the photographs were made a month later, *i. e.*, while the plant was still young.

Fifteen plants were inoculated and only one gave a fasciated shoot. All depends, apparently, on whether the needle hits or misses the dormant bud. Most of the other plants, however, developed a shoot, often a strong one, from the dormant bud in the inoculated axil *and from that bud only*, showing very strikingly the effect of the tumor stimulus.

A photograph of one of these non-fasciated tumor-stimulated shoots made on January 14, *i. e.*, two months after the inoculation, is shown on plate XVIII at 2, the subtending petiole *P* being now dead.

In the paper on Bryophyllum, I called attention to a certain resemblance of the stimulated shoots to the witch-brooms of "peach yellows," our most destructive peach disease, the cause of which remains unknown, but in which we know that the tree has difficulty in translocating its starch. In that disease the summer sprouts which appear on the trunk and main limbs develop feeble branches in their leaf axils the same season, and so on, until within a space of 5 or 6 months, say from June to November in the latitude of Delaware, 3 sets of weak branches may develop from leaf axils on such shoots, all or most of these shoots dying the following season, at least at the top. It would seem that the proximate cause of the two diseases must be the same, to wit, stimulus of excessive amounts of water and foodstuffs acting locally as the result of phloem injury.

In Bryophyllum, tertiary shoots developed within a few weeks from the leaf axils of the crown-gall stimulated, hasty, secondary shoots. In the nasturtium plant shown in plate XVIII, this process has been carried



one step farther, as far or almost as far as it ever goes in peach yellows, *i. e.*, the inoculated leaf axil of the primary shoot (here designated as 1) has given rise to a secondary shoot (2), this in turn has given rise (always from leaf axils) to two well developed tertiary shoots (3, 3), and these in turn have given rise to four quaternary shoots, all within a period of two months.

It is a remarkable thing that within so short a time a crown-gall inoculation should have given rise to 3 sets of shoots and it can be explained only on the supposition that the growth of the tumor caused a powerful centripetal movement of water and foodstuffs which also acted on the adjacent dormant bud (mother of 2) and continued to act excessively on the shoot which developed from it. This is shown also by the fact that the tertiary shoots (3, 3) are twice the diameter of the main axis (1). The terminal leaves on the shoots 2 and 3, 3, were wilted when the plant was brought in from the hothouse for photographing and as the plants were free from insects and otherwise under good conditions, and as all the checks remained normal, I had no way of explaining this except to suppose that the shoots, excessively nourished at first, were finally unable to get enough water through their vascular system, owing to its invasion or compression by the axillary tumor. This is the usual course of events and shoots growing out of crown galls generally die early. Cross sections in series were afterwards made from the base of branch 2 and this hypothesis was confirmed. Very likely the downward movement of elaborated food stuffs (sugar and proteids) in this shoot (2) was also eventually interfered with by the growth of the tumor and that too considerably earlier than the shutting off of the water supply, so that the tumor has acted as a stimulus to the growth of the shoot in both a positive and a negative way: positive by attracting food to itself some of which was also used by the shoot; negative by interfering, through gradually increasing compression, with the normal nightly downward movement of elaborated carbohydrates.

Shoot No. 2 (Pl. XVIII) was not tested for starch because I did not then have it in mind, but since the preceding paragraph was written, I have found (April 13-14) 3 nasturtium plants of a later inoculation (Jan. 20 1922), which show the same phenomenon, *i. e.*, shoots pushing from the axil bearing the tumor and a marked thickening of these shoots some distance away from the tumor *i. e.*, to more than twice the diameter of the main axis, as in Pl. XVIII, 3, 3, with wilting of terminal leaves and in each instance thin cross-sections of the base of the shoots treated with iodine showed in the cortex, pith and medullary rays a marked accumu-

lation of starch, very much more than in the main axis. Here, clearly, the constriction of the base of the shoot by interfering with the normal downward movement of the elaborated foodstuffs was the cause of the thickening of its axis and of the pushing of its dormant buds, and further constriction caused the wilting. The location of the enlargement of the shootaxis at some distance from the tumor shows also that for some time, in each case, there was no compression of the base of the shoot. In one of these plants a branch corresponding to 4 of plate XVIII had a so pushed buds, making four sets of shoots in less than three months.

In plate XIX, made from a freehand section treated with iodine, may be seen the abnormal storage of starch in the cortex of one of the swollen axillary shoots referred to in the preceding paragraph. The constricting tumor was about one centimeter below this point. Plate XX is from the pith of another plant in the same series. Here also the shoot was constricted by the tumor and the downward movement of sugar and proteids was interfered with.

In this disease, therefore, we may have all varieties of stimulating secondary effects on normal tissues from prolepsis of uninjured leaf and flower buds and root anlage located in the vicinity of the tumors, through simple fusions or divisions (fasciations), to the breaking up of the dormant bud, or of a cambium, into dozens and even hundreds of small vegetative fragments which may either grow as roots or shoots on the surface of the tumor or be buried in its depths. Due to the same organism we may have, therefore, both organoid and histioid galls although these are at the two poles of Küster's classification. The tumor, of course, also exerts purely destructive effects on surrounding tissues but these do not concern us here.

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#### DESCRIPTION OF PLATES XVI TO XX

PLATE XVI. A. Hop strain of crown-gall organism (Sunflower Colony 1) inoculated November 17, 1921, in a lower leaf axil of *Tropaeolum majus* L., by means of needle pricks. Result: A small tumor, not clearly visible here, above which is a fasciated shoot—the only secondary shoot on the plant. Photographed December 19, 1921. About one-third natural size. Actual size of the twisted, flattened shoot,  $1\frac{3}{4}$  inches by  $\frac{1}{4}$  by  $\frac{1}{6}$  inch, the last two measurements being in the middle part.

PLATE XVI. B. Base of the fasciated shoot. Stub of the subtending petiole (now

dead) at *P*. Primary tumor at *T*, *T*. A downward extension of this tumor has burst through the cortex of the main axis (*M*) at *S*. A shoot is pushing in the leaf axil *X*, and another was pushing from the axil of a dwarfed leaf on the back side of the base. There are incipient roots at *R*. At *F* there is a third secondary (fasciated) axis of growth, the other two being at the apex of the shoot (Pl. XVII). On sectioning, the tumor tissue was found to have extended upward in the vascular region of the shoot as far as indicated by the arrow.  $\times 5$ .

PLATE XVII. Upper part of fasciation on plate XVI. *B*, showing two axes of growth, *A*, *B*. This part is quite flat. Main axis of the plant at *M* with a petiole *p* at its right. Buds were pushing from three visible leaf axils *X*, *X*, *X*, and also on the back side from the axil of leaf *L*.  $\times 5$ .

PLATE XVIII. Inoculated axillary crown gall on nasturtium: 1, main axis unbranched except as shown in the photograph. *P*, petiole in the axil of which the plant was inoculated by needle pricks November 17, 1921, *t*, tumor that developed; *P* 2, secondary shoot derived from an uninjured axillary bud growing as a result of the tumor stimulus; 3, tertiary shoots, very plump; 4, quaternary shoots. Leaves wilting from late interference with water-supply. Photographed January 14, 1922.  $\frac{3}{4}$  natural size.

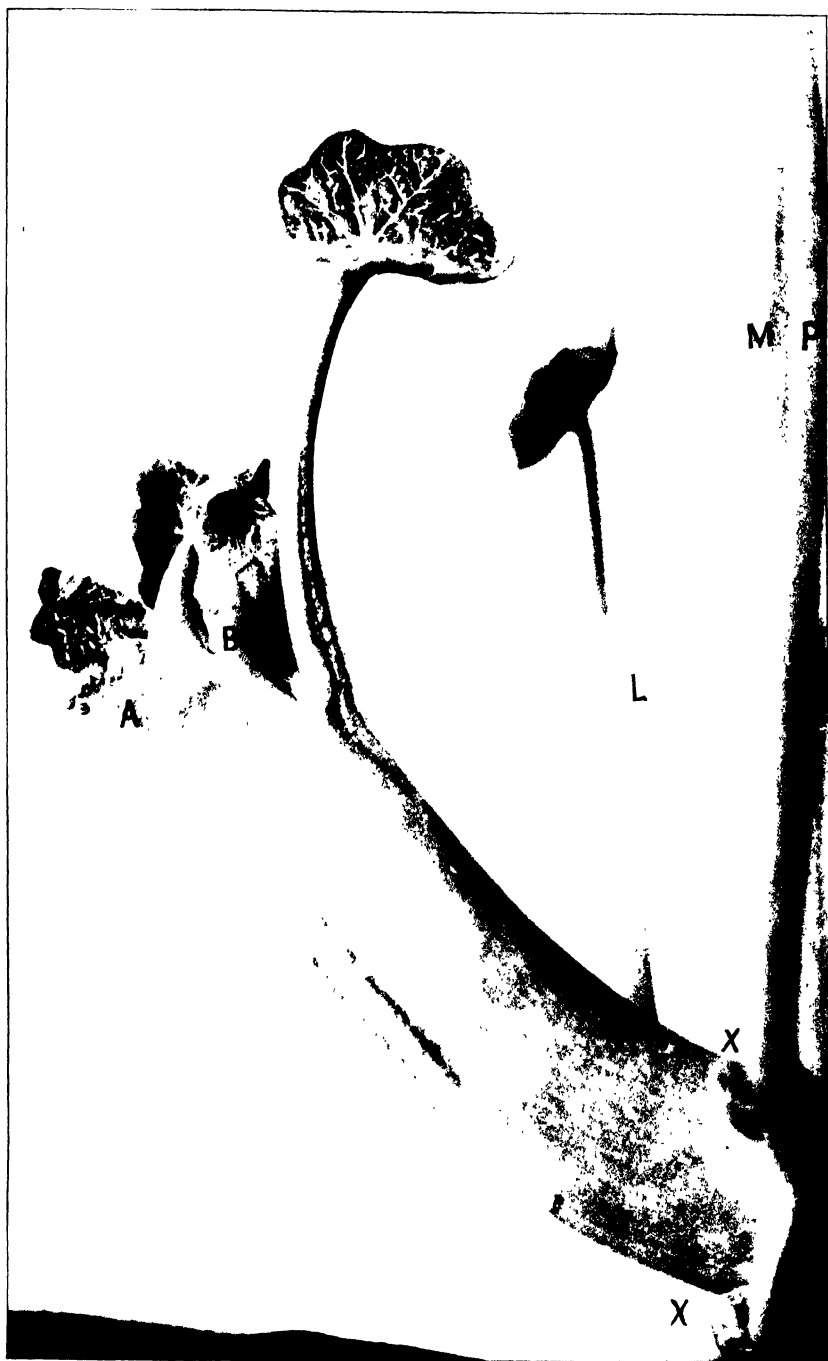
Pl. XIX. Starch in cortex in a swollen axillary nasturtium shoot collected in the morning. Translocation blocked by tumor in same axil. The whole cortex was like this.

Pl. XX. Starch in pith in a swollen axillary nasturtium shoot collected in the morning. Translocation blocked by tumor in the same axil. The whole pith was like this; also many of the medullary rays. Not the same plant as that shown in Plate XIX.



CROWN-GALL INOCULATIONS





CROWN-GALL INOCULATIONS



SMITH: FASCIATION AND PROLEPSIS



CROWN-GALL INOCULATIONS





SMITH: FASCIATION AND PROLEPSIS



CROWN-GALL INOCULATIONS



SMITH: FASCIATION AND PROLEPSIS



CROWN-GALL INOCULATIONS



# PATHOGENICITY OF THE OLIVE KNOT ORGANISM ON HOSTS RELATED TO THE OLIVE.<sup>1</sup>

CLAYTON O. SMITH

WITH PLATES XXI AND XXII

## INTRODUCTION

The bacterial organism *Pseudomonas savastanoi* E. F. S., has been carefully studied by different investigators and its pathogenicity on the olive fully determined. Its pathogenicity, however, on other closely related hosts has not been definitely proved. Savastano<sup>2</sup> on inoculating *Nerium oleander*, *Osmanthus* (*Olea*) *fragrans*, grapes, peaches, plums, almonds, figs, apples and pears, with pure cultures of the olive-knot organism as well as with olive-knot tissue obtained negative results. Smith<sup>3</sup> also reports negative results in his inoculations on the oleander, *Chrysanthemum frutescens*, several species of *Fraxinus*, privets and other plants more or less closely related to the olive. On one host, *Fraxinus ornus*, slight swellings were visible, but the experiment was terminated by an accident before the results were fully determined.

## PLAN OF EXPERIMENT

The experiments on which this paper is based extended through the years 1919 to 1921 in the course of which plants more or less closely related taxonomically to the olive were inoculated. Pure cultures of *Pseudomonas savastanoi* isolated from the typical olive knot and incubated at 25° C. for from 24–48 hours were used. Ten inoculations or multiples of ten with control punctures were made at the same time on each host. The olive was included in order to check the virulence of the culture used. A steel needle was employed in the inoculation work and no protection was given the punctures after they were made. The data were taken at the close of the growing season usually in December of the year the experiment was made.

The following species were inoculated: olive (*Olea europaea* Linn.), *Fraxinus velutina* Torr., *F. floribunda* (S. P. I. 47687) which closely

<sup>1</sup> Paper No. 88. University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

<sup>2</sup> Savastano, L. Sulla trasmissibilità del bacillo della tubercolosi dell' olivo nell' oleandro. Bol. Arbor. Ital., 4: 86–87. 1908.

<sup>3</sup> Smith, Erwin F. Recent studies of the olive-tubercle organism. U. S. Dept. Agr. Bur. Plant Industry Bul. 131, part IV, p. 25–43. 1908.

resembles *F. Ornus* Linn., *Adelia* (Forestiera) *acuminata* Michx., privet (*Ligustrum ovalifolium* Hassk.), *Chionanthus virginica* Linn., *Osmanthus fragrans* Lour., *Osmanthus* (*Olea*) *aquifolium* Sieb., *Vinca*, *Thevetia nereifolia* Juss., *Nerium oleander* Linn., *Coprosma baueri* Endl., *Carissa grandiflora* DC., *Chrysanthemum frutescens* Linn., *Elaeagnus angustifolia* Linn., lilac, and jasmine (probably *Jasminum primulinum* Hensl.) and several species of *Prunus* that have proved especially susceptible to *Pseudomonas tumefaciens* Sm. and T.

#### RESULTS

The tests for 1919 were doubtful except on *Adelia* and privet. Tests made the following two years proved other hosts to be susceptible, as shown by typical knots and less evident response in the form of definite lesions. The various hosts reacted somewhat differently. On some of the hosts the results were negative; on others only slight hypertrophies developed at the margin of the wound, which in some cases eventually gave rise to a definite canker of some size. On still other hosts as illustrated in plate XXI subspherical knots were produced closely resembling those of the olive. These results will be considered more fully under each of the hosts. Of the hosts mentioned above the following gave negative or doubtful results: *Osmanthus fragrans*, *Vinca*, *Thevetia nereifolia*, *Nerium oleander*, *Coprosma baueri*, *Carissa grandiflora*, *Chrysanthemum frutescens*, *Elaeagnus angustifolia*, lilac, and species of *Prunus*. Privet gave negative results except in the tests of 1919, where small knob-like growths developed, (Pl. XXI, fig. 16). *Osmanthus aquifolium* and *Chionanthus virginica* gave definite lesions. The hosts which gave knots or galls were *Fraxinus velutina*, *F. floribunda*, *Adelia acuminata*, *Jasminum primulinum*.

*Adelia acuminata* (Tab. I) developed typical knots that closely re-

TABLE 1  
*Inoculations on Adelia acuminata*

Date	Punctures	Number <sup>1</sup>	Diameter in mm.
June 28, 1919.....	10	6	5-15
Aug. 4, 1919.....	10	8	2-4
Aug. 12, 1919.....	10	6	2-3
Apr. 15, 1920.....	10	6	10-15
Apr. 23, 1920.....	10	6	3-8
June 15, 1920.....	10	9	4-8
June 21, 1920.....	10	7	1-2
July 16, 1920.....	10	8	2-3

<sup>1</sup> In Tables 1 to 5 inclusive this column indicates the number of hypertrophies which developed.

sembled those of the organism on olive, but were somewhat smaller, being from 2-15 mm. in diameter (Pl. XXI, figs. 13-14). They were somewhat irregular in shape and protruded from the normal tissue as hypertrophies. Section of the knots showed definite bacterial cavities containing numerous bacteria. This host is closely related to the olive and has been reported to have been successfully grafted to it.

*Fraxinus velutina* and *F. floribunda* (the latter a small plant sent from the United States Department of Agriculture, Bureau of Seed and Plant Introduction as number 47687) were tested (Tab. 2). The latter species is native to India and much resembles *F. Ornus*, the species used by Dr. Erwin Smith in his experiment, where small enlargements developed about the punctures.

TABLE 2  
*Inoculations on Fraxinus*

Date	Species	Punctures	Number	Diameter in mm.
Mar. 4, 1920.....	Velutina	10	6	4-8
Mar. 11, 1921.....	"	20	6	2-11
Mar. 21, 1921.....	"	30	18	2-5
Mar. 21, 1921.....	"	10 check	0	
Apr. 13, 1921.....	"	10	9	4-5
Apr. 13, 1921.....	"	10 check	0	4-5
May 5, 1921.....	Floribunda	5	5	4-10

In both of the above species galls were formed at punctures in the young succulent stem and on the petioles of leaves (Pl. XXI, fig. 2). The knots of *F. velutina* were still fresh and green a year after the inoculation (Pl. XXI, fig. 1). These have made but little new growth during the second season, but pointlike elevations have appeared that in some instances extend 2 mm. beyond the general surface of the knot. These knots (Pl. XXI, fig. 3-4) seem to form readily upon inoculation of actively growing tissue. The inoculations made early in the season were the best developed while those made late in the season (after June) were generally unsuccessful.

*Chionanthus virginica* (Tab. 3) when inoculated with the olive knot organism did not form typical knots except on the leaves (Pl. XXI, figs. 10-11), but it did produce definite lesions (Pl. XXI, figs. 8-9). The first stages showed a water-soaked, brownish area above and below the puncture. In two weeks a slight swelling developed around the punctures (Pl. XXI, fig. 12). This hypertrophied tissue was of a lighter green color than normal tissue. At this time the original puncture had developed into an enlarged depressed spot, caused by a slight killing of the tissue.



TABLE 3

*Inoculation on Chionanthus virginica*

Date	Punctures	Number <sup>1</sup>	Diameter in mm.
Apr. 10, 1920.....	10	6	3-7
Apr. 30, 1920.....	20	11	4-15
June 15, 1920.....	20	15	4-15
July 16, 1920.....	20	11	3-12
July 26, 1920.....	20	13	3-25
Apr. 13, 1921.....	20	18	10-15
Apr. 13, 1921.....	10 check	0	
Apr. 29, 1921.....	10	3	5-13

Definite scars or lesions eventually developed at the point of inoculation, with hypertrophy of tissue at their margins. These lesions continued to increase in size with the growth of the host. Their surfaces were covered with a dark hypertrophied growth with numerous fissures. Inoculation of young tissue frequently resulted in a modification of its growth by causing a shortening and thickening of the shoot (Pl. XXI, fig. 9). The abnormal hypertrophies, however, developed more often within the normal limits of the tissue than as an external knot. Inoculation in the veins and petioles of the leaf showed positive results in lesions in the twisting of the leaf and at length in the production of small galls (Pl. XXI, fig. 10).

*Osmanthus aquifolium* when inoculated with the olive knot organism (Tab. 4) did not develop any large external knots. The enlargement of tissue generally involved the regions immediately surrounding the puncture (Pl. XXI, fig. 6). The healing tissue at the margin of the puncture sometimes rounded up in an abnormal manner as compared with control punctures. In some instances very small (3-4 mm.) round shaped galls developed (Pl. XXI, fig. 7). Their growth did not continue, however, and was only slightly suggestive of the initial olive knot. The tissue that developed about the punctures was of a darker color and rougher than normal callous tissue. The olive knot organism was successfully cultured from the hypertrophied tissue of this knot (Pl. XXI,

TABLE 4

*Inoculations on Osmanthus aquifolium*

Date	Puncture	Number	Diameter in mm.
June 18, 1920.....	20	10	4-8
June 21, 1920.....	20	12	3-6
July 16, 1920.....	10	5	6-15
May 16, 1921.....	30	16	3-5

<sup>1</sup> The hypertrophies that here develop were more in the nature of lesions than knots.

fig. 7) 6 months after inoculation. The hypertrophied tissue at length dies becoming a dark color and very hard and dry.

More recent inoculations have given more typical knots (Pl. XXI, fig. 5) which were more like those produced in *Adelia* and *Fraxinus*.

On *Jasminum primulinum* (Tab. 5) definite and characteristic knots developed (Pl. XXI, fig. 15). These first began to form around punctures at the margin of the wounds as aerial hypertrophies which were at first of a greenish color but later became hard and dried and of a darker color. Little hypertrophy if any took place within the normal tissue although some darkening of tissue could be observed in the pith (Pl. XXII, fig. 19).

TABLE 5

*Inoculations on Jasminum primulinum*

Date	Punctures	Number	Diameter in mm.
Jan. 31, 1921.....	20	1	2
Feb. 21, 1921.....	10	7	3-5
Nov. 11, 1921.....	20	7	3-5
May 16, 1921.....	10	9	3-5
June 27, 1921.....	20	18	3-9
July 18, 1921.....	10	8	2-5
July 29, 1921.....	10	7	2-7

## REISOLATION OF ORGANISM

The causal organism isolated from the artificial knots on the various hosts was then used for inoculation on the olive (Tab. 6.) Reisolations were made from *Jasminum*, *Fraxinus*, *Olive*, *Chionanthus*, *Adelia* and *Osmanthus*. When these organisms were inoculated into the olive, typical olive galls developed except with *Fraxinus*. The organisms isolated appeared to resemble the olive-knot organism on glucose potato agar and other media.

## PATHOLOGICAL HISTOLOGY

The olive-knot organism is characterized by the production of masses of bacteria in definite cavities within the hypertrophied tissue of the olive. This is a very evident difference from galls of *Pseudomonas tumefaciens* on various hosts where very few organisms are present within the hypertrophied cells.

Microtome sections of the knots of the various hosts giving positive results with *Pseudomonas savastanoi* were examined microscopically. These were cut with a sliding microtome after being fixed in Carnoy's solution. They were stained with acid fuchsin. In general the sec-

TABLE 6

*Reisolation of Pseudomonas savastanoi*

Date of Inoc.	Host inoculated	Date of reisolation	Result of reinoculation of olive	
			Number <sup>1</sup>	Size (mm.)
June 9, 1920.....	Chionanthus	Dec. 27, 1920	(20)19	5-10
June 18, 1920.....	Osmanthus	Dec. 28, 1920	(20)15	2-5
July 16, 1920.....	Adelia	Dec. 28, 1920	(20)15	3-12
June 21, 1920.....	Olive	Feb. 15, 1921	(20)19	5-10
Mar. 21, 1921.....	Fraxinus	July 18, 1921	(40)0	
Apr. 13, 1921.....	Chionanthus	May 15, 1921	(40)27	5-25
May 16, 1921.....	Osmanthus	Aug. 16, 1921	(20)15	2-3
May 27, 1921.....	Chionanthus	Aug. 16, 1921	(20)20	2-12
June 27, 1921.....	Jasminum	Aug. 20, 1921	(10)8	3-8

tions of these knots from different hosts showed the same characteristics as the typical olive knot. The organism occurs in mass and is easily demonstrated microscopically. These masses are usually associated with tissue that gives a more brownish stain than the rest. Sections of the knot on the midvein of Chionanthus (Pl. XXII, figs. 17-18) in the center of which was a large cavity, were studied. The presence of bacteria was readily demonstrated near the margin where they had not been washed away by the process of staining the sections.

TABLE 7

*Results of the inoculation of different hosts with Pseudomonas savastanoi. The number in parenthesis is the number of knots that developed from 10 punctures. The other numbers give the size of the knots in millimeters*

Date	Olive	Fraxinus	Adelia	Chionanthus	Osmanthus	Privet	Jasmine
June 28, 1919	(10)8-20	—	(6)5-15	—	—	(9)2-5	
Aug. 4, 1919	—	—	(8)2-3	—	—	(2)2-3	
Apr. 30, 1920	(4)12-20	—	(6)10-15	0	—	0	
June 15, 1920	(10)8-10	0	(9)4-8	(9)8-10	—		
June 21, 1920	(10)6-10	0	(7)1-2	(6)4-6	—		(1)2
Jan. 31, 1921	(5)6-10	—	—	—	—		(7)3-5
Mar. 11, 1921	(5)6-10	(3)48	—	—	—		—
Mar. 21, 1921	(4)2-5	(9)2-11	—	—	—		—
Apr. 13, 1921	—	(9)4-5	—	(8)10-15	—		—
May 5, 1921	(6)2-5	(5)4-10	—	—	—		
June 27, 1921	(7)3-12	—	—	(9)3-5	(18) <sup>3</sup> 3-9		
July 29, 1921	(10)6-10	—	—	—	(7)2-7		

<sup>1</sup> The number in parenthesis denotes the number of puncture inoculations, the one outside the parenthesis the number of knots that developed.

<sup>2</sup> This host is *Fraxinus floribunda*, all others *F. velutina*.

<sup>3</sup> In this test 20 punctures were made.

Sections of *Jasminum primulinum* still showed the old needle puncture through the stem, the path of the needle being filled with hypertrophied tissue which is especially noticeable in the pith and in the xylem region (Pl. XXII, fig. 19).

In certain parts of this needle path can be found numerous organisms. Masses of bacteria were also found in the hypertrophied tissue, especially near those parts that take the reddish-brown stain. The hypertrophy in Jasmine was parenchymatous in nature, but in these knots, vascular elements were often present although usually displaced from their normal position.

#### SUMMARY AND DISCUSSION

1. Typical artificial knots have been produced with pure cultures of the olive-knot organism on *Adelia* and two species of *Fraxinus*. These more or less closely resemble the knot produced on the olive by the same organism.

2. The inoculations on *Chionanthus* and *Osmanthus* do not usually result in the production of galls or knots, yet there is a very positive pathological effect, especially a stimulation of the growth of tissue followed often by a slight necrosis.

3. The size of the hypertrophies (knots) produced on the hosts mentioned in 1 and 2 were smaller than those on the olive, with the possible exception of some on *Fraxinus floribunda*. They apparently reach their maximum size in 3 or 4 months after which the tissue gradually dies. This seems to take place sooner than in the knot on the olive.

4. The olive-knot organism has been regarded as parasitic only on the olive. These experiments suggest that it may not be so restricted, but that it may possibly occur naturally elsewhere, especially on certain species of *Fraxinus*.

5. The organism in no case was infectious in hosts not related taxonomically to the olive. It seems to be restricted to plants closely related botanically to the olive, and especially to those of the family Oleaceae.

6. The limited pathogenicity of the olive-knot organism would seem to separate it definitely from *Pseudomonas tumefaciens*.

#### DESCRIPTION OF PLATES

PLATE XXI. Results from artificial inoculation with *Pseudomonas savastanoi* on hosts related to the olive.

FIGS. 1-4. *Fraxinus*. (1) *F. velutina*, inoculated May 4, photographed Dec. 1

1920;<sup>1</sup> (2) *F. floribunda*, two puncture inoculations on stem and two on leaf petioles, May 5, Aug. 5, 1921; (3) *F. velutina* Mar. 21, July 1, 1921; (4) Mar. 11, *F. velutina* Nov. 1, 1920.

FIGS. 5-7. *Osmanthus aquifolium*. (5), Inoculation with olive-knot tissue, July 1, 1921, Jan. 1, 1922; (6), 4 positive puncture inoculations on stem and 7 on leaves, May 16, Aug. 16, 1921; (7) 3 puncture inoculations made on same host, at same time and from same culture as figure 6.

FIGS. 8-12. *Chionanthus virginica*. (8) Advanced cankers such as develop from artificial inoculations on older shoots, June 12, Dec. 12, 1921; (9) results in rapidly growing succulent tissue, from inoculation, July 15, Sept. 15, 1920; (10)-(11), puncture inoculations of leaf parenchyma and midvein, May 26, Nov. 1, 1921; (12), lesions in their early stages of development, showing the abnormally thickened margin, Apr. 3, May 24, 1921.

FIGS. 13-14. *Adelia (Forestiera) acuminata*. (13), 3 knots on stem, June 30, Dec. 1, 1921; (14), nearly spherical knots, Apr. 15, Dec. 15, 1920.

FIG. 15. *Jasminum primulinum* puncture inoculated, June 27, Dec. 1, 1921.

FIG. 16. *Ligustrum ovalifolium* (Privet). Very small hypertrophies or knob-like growth, the only ones produced, although other inoculations were made at various times, June 28, Dec. 1, 1919.

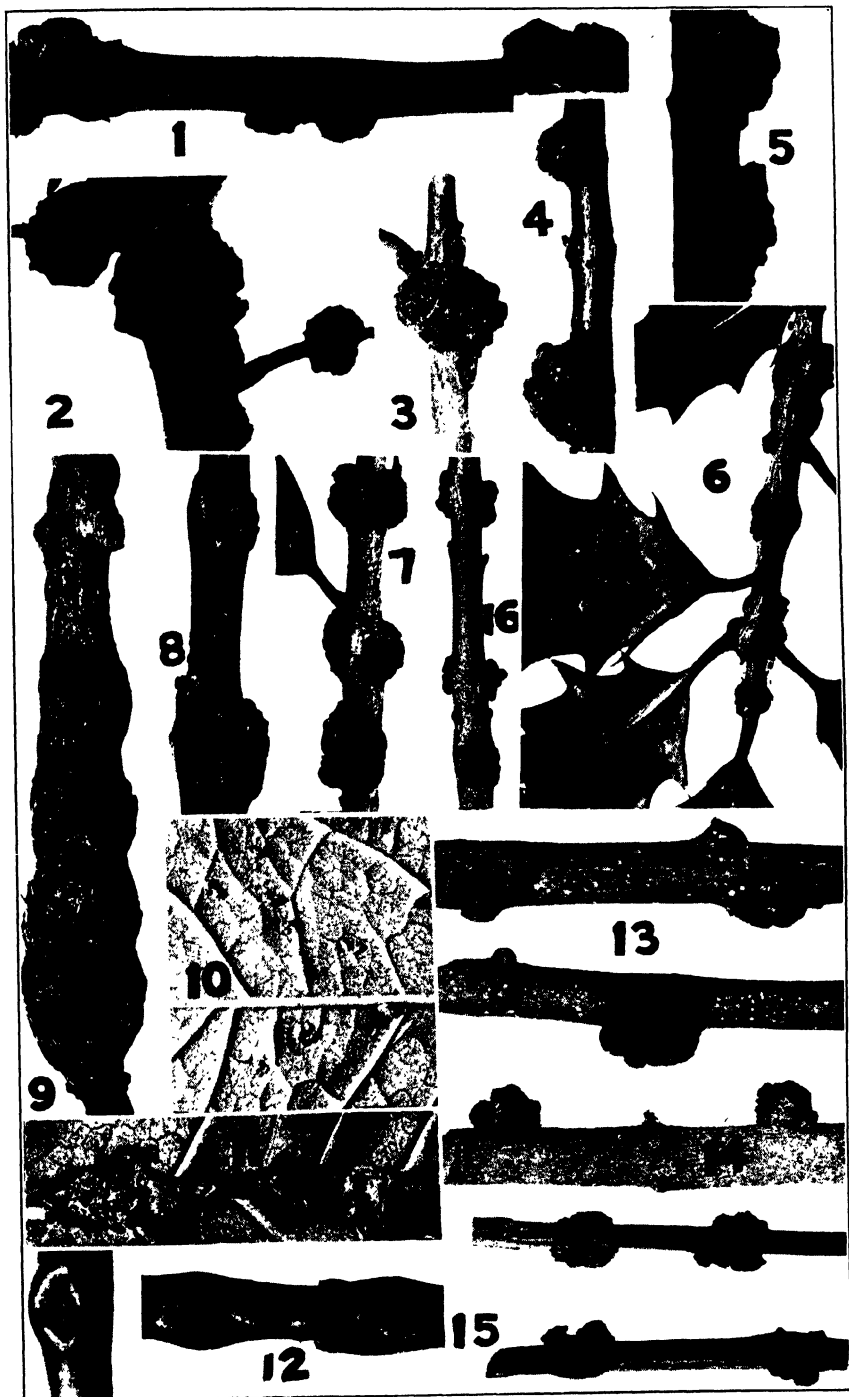
#### PLATE XXII. Cross Sections of Artificial Knots.

FIGS. 17-18. These two figures represent a single cross section, that was cut in two parts for arrangement on plate. They represent a cross section of knot at right on midvein, (Fig. 11) magnified about 15 diameters. The spaces have numerous bacteria in mass near the margins, especially the space shown in figures 17-18. The adjacent tissue is also invaded. The upper space of figure 18 has bacteria, but fewer in number. The black crosses in a general way indicate where the masses of bacteria are found. It is probable that both of the spaces were filled with the organisms which were largely washed away in staining and mounting.

FIG. 19. Cross section of knot of *Jasminum primulinum* similar to those of figure 15 magnified about 14 diameters. The crosses indicate approximately where the masses of bacteria are located. In the center is a mass of hypertrophied tissue partially surrounded by normal pith cells.

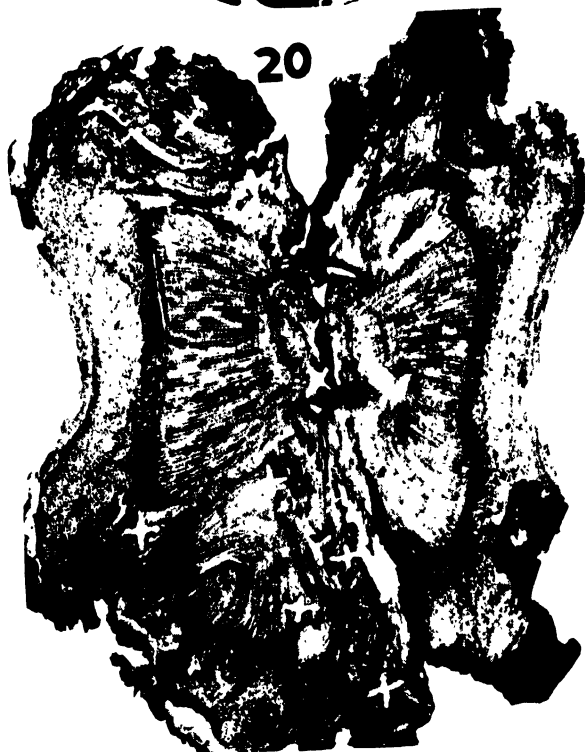
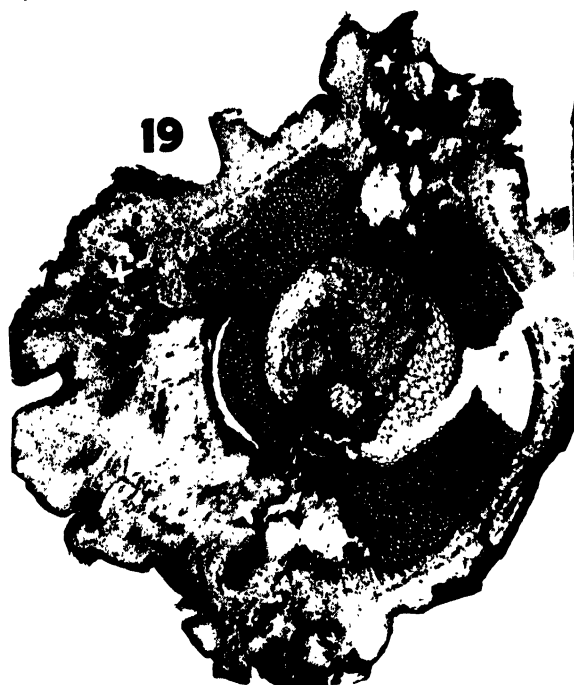
FIG. 20. A cross section of middle knot of figure 7 magnified about 12 diameters. The white crosses indicate the approximate location of the larger bacterial masses, which often take a deeper stain. The path of the inoculating needle is still evident.

<sup>1</sup>In the following legends the first date is that of the inoculation, the second the time when photographed.



OLIVE KNOT ON HOSTS RELATED TO THE OLIVE





CROSS SECTIONS OF HYPERTROPHIES





# ON THE USE OF THE ACETATES OF COPPER AS FUNGICIDES.

O. BUTLER AND T. O. SMITH

WITH ONE FIGURE IN THE TEXT

Two acetates of copper are used as fungicides, the normal or neutral acetate of copper  $\text{Cu}(\text{C}_2\text{H}_3\text{O}_2)_2$ ,  $\text{H}_2\text{O}$  and the basic acetate of copper  $\text{Cu}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot \text{CuO} \cdot 6\text{H}_2\text{O}$  the former containing 31.8 per cent. metallic copper, the latter 34.4 per cent. Basic acetate of copper was introduced by Bencker<sup>1</sup> in 1889 and is still used especially by the grape growers of meridional France; neutral acetate of copper has been employed, according to Chuard and Dusserre<sup>2</sup>, since 1892 in France at the Ecully School, and Briosi<sup>3</sup> has also used it with excellent results for the control of *Plasmopara viticola* in Italy. In the United States the copper acetates are practically unknown and we hardly find mention of them in the literature. Neutral acetate of copper was used by Galloway<sup>4</sup> in 1890 for the control of *Guignardia bidwellii*, and, at a strength of 1.13 per cent, gave the equivalent control of 3.4 per cent. Bordeaux mixture 1:0.66. Neutral acetate of copper has also been used as a spray for *Cylindrosporium padi* on plum at a strength of 0.078 per cent and 0.156 per cent respectively<sup>5</sup> for *Entomosporium maculatum* on quince at strengths of 0.1 per cent, and 0.2 per cent.; for *Guignardia bidwellii* *Entomosporium* and scab on pear at 0.078 per cent.<sup>6</sup> In the case of the experiments with *Cylindrosporium padi* the acetate was compared with 0.078 per cent Bordeaux mixture 1:7.5 and certain other fungicides but in "none of the experiments could any very perceptible difference be noted between treated and untreated trees."<sup>7</sup> In the experiments with *Ento-*

<sup>1</sup> Bencker, Georges. Traitement du mildiou. Prog. Agric. et Vitic. 12: (An. 6): 90-94. 1889.

<sup>2</sup> Chuard, E. and C. Dusserre. Sur les verdets employés dans la lutte contre le mildiou. Chron. Agr. Vaud 17: 291-297. 1904.

<sup>3</sup> Briosi, Giovanni. Esperienza per combattere la Peronospora della vite. Att. 1st. Bot. Univ. Pavia (Ser. 2) 4: 149-154. 1897.

<sup>4</sup> Galloway, B. T. Experiments in the treatment of plant diseases. Jour. Mycology 7: 12-16. 1894.

<sup>5</sup> Galloway, B. T. Report on the experiments made in 1891 in the treatment of plant diseases. United States Dept. Agric. Div. Veg. Path. Bull. 3. 70 p. 1892.

From the method of preparation of the fungicide mentioned in the text (page 65) it is not likely that basic copper acetate was used.

<sup>6</sup> Loc. cit. supra, pages 65 and 10.

<sup>7</sup> Loc. cit. p. 63.

*mosporium maculatum* the acetates were compared with 0.103 per cent Bordeaux 1:1.33 the following results being obtained: The trees sprayed with the Bordeaux mixture produced 42.9 per cent more clean fruit than the non-sprayed trees; the 0.1 per cent neutral acetate produced 40.8 per cent more clean fruit than the non-sprayed trees, and the 0.2 per cent wash 44.9 per cent more clean fruit. In the experiments with *Entomosporium* and scab the 0.625 per cent Bordeaux mixture 1:0.66 gave in the case of *Entomosporium* 21.2 times better protection than no spraying at all, whereas in the case of scab the amount of disease present was small and the copper acetate and Bordeaux mixture afforded a similar degree of protection. In the experiments on the control of *Guignardia bidwellii* 0.625 per cent Bordeaux mixture 1:0.66 was used but despite this fact the neutral copper acetate yielded a protection scarcely inferior to that obtained with the Bordeaux mixture.

Not only have the copper acetates been but little employed in the United States but formulae for their use are rarely seen in texts dealing with fungicides though curiously enough one generally finds a formula given for a cuprammonium. The cuprammoniums are used when a fungicide forming very inconspicuous spots on foliage or fruit is desired and yet the acetates of copper form even less conspicuous deposits, and, for the equivalent in copper, are many times less injurious to the plant sprayed than the former. In fact, and any one who so desires can readily verify the statement for himself, the acetates of copper are, when sprayed under conditions that permit drying of the foliage within an hour after the fungicide has been applied, no more toxic than a 1 per cent Bordeaux mixture, while it is unsafe to use cuprammonium sulphate (eau céleste), the least toxic of the cuprammoniums, at a greater strength than 0.25 per cent.<sup>1</sup> Furthermore, the acetates of copper are more adhesive than the cuprammoniums<sup>2</sup> and cost no more or but little more per equivalent of copper than cuprammonium sulphate which is the cheapest of the cuprammonium washes.

The evidence is abundant that the acetates of copper compare favorably with Bordeaux mixture in fungicidal properties. Foëx<sup>3</sup> tells us that the experimental trials made with basic copper acetate at the agricultural school of Montpellier have consistently given as satisfactory

<sup>1</sup> Butler, O. The cuprammonium washes. *Phytopathology* 7: 235-268. Pl. 3-10. 1917. Bibliography, p. 267.

<sup>2</sup> Butler, O. and T. O. Smith. Relative adhesiveness of the copper fungicides. *Phytopathology* 9: 431-444. 1919. Literature cited, p. 444.

<sup>3</sup> Foëx, Gustave. *Cours complet de Viticulture*. 4th ed. p. 579. Montpellier. 1895.

control as Bordeaux mixture and have even shown the former to possess greater adhesive properties. Viala<sup>1</sup> tells us that the numerous comparative experiments made during the last few years have shown clearly and unmistakably that basic acetate of copper washes constitute one of the most perfect methods of treatment of the downy mildew.

"For the last twelve years," Pacottet<sup>2</sup> emphatically remarks, "I have used it in Burgundy and have never seen the downy mildew. My grapes are the last to lose their leaves and some day I will show that it occupies the first rank in harmlessness to vegetation."

The acetates of copper, says Marsais,<sup>3</sup> are being increasingly used in viticulture.

Neutral acetate of copper was extensively used in the Canton de Vaud in 1904 and gave as satisfactory control as Bordeaux Burgundy mixture.<sup>4</sup>

Furthermore, the acetates of copper are non-toxic to the plant to be protected and form less conspicuous spots than the cuprammoniums. When we say that the acetates are non-toxic to the plant to be protected the reader should understand that we mean by this that they are non-toxic to the plant to be protected if the plant in question will stand spraying with Bordeaux mixture in which the ratio copper to lime is unity or less, but if Bordeaux mixture is injurious then the acetates will prove injurious also and may be found more injurious than 0.5 per cent or stronger Bordeaux mixture when used at equivalent strengths in copper. The acetates of copper are excellent fungicides. They compare favorably with Bordeaux mixture as regards efficiency and effectiveness and, while possessing all the fungicidal properties of the cuprammoniums, are not as toxic to the plant to be protected. For these reasons, therefore, we have thought it desirable to call attention to the copper acetates and particularly to the desirability of substituting them for the cuprammoniums.

Neutral copper acetate dissolves readily in cold water forming a clear bluish-green solution with faint acetic odor. When sprayed upon a plant the salt already in unstable equilibrium decomposes with the formation

<sup>1</sup> Viala, Pierre. *Les maladies de la vigne*. 3rd ed. p. 143 et seq. 1893.

<sup>2</sup> Pacottet, P. *Sur le traitement du mildiou*. *Rev. Vitic. (An. 14)* 27: 489-492. 1907.

<sup>3</sup> Marsais, P. *Traitement du mildiou*. *Rev. Vitic. (An. 15)* 29: 595. 1908.

<sup>4</sup> Chuard, E. and H. Faes. *Enquete sur l'emploi du verdet neutre dans le vignoble vaudois en 1904*. *Chron. Agr. Vaud* 17: 601-608. 1904.

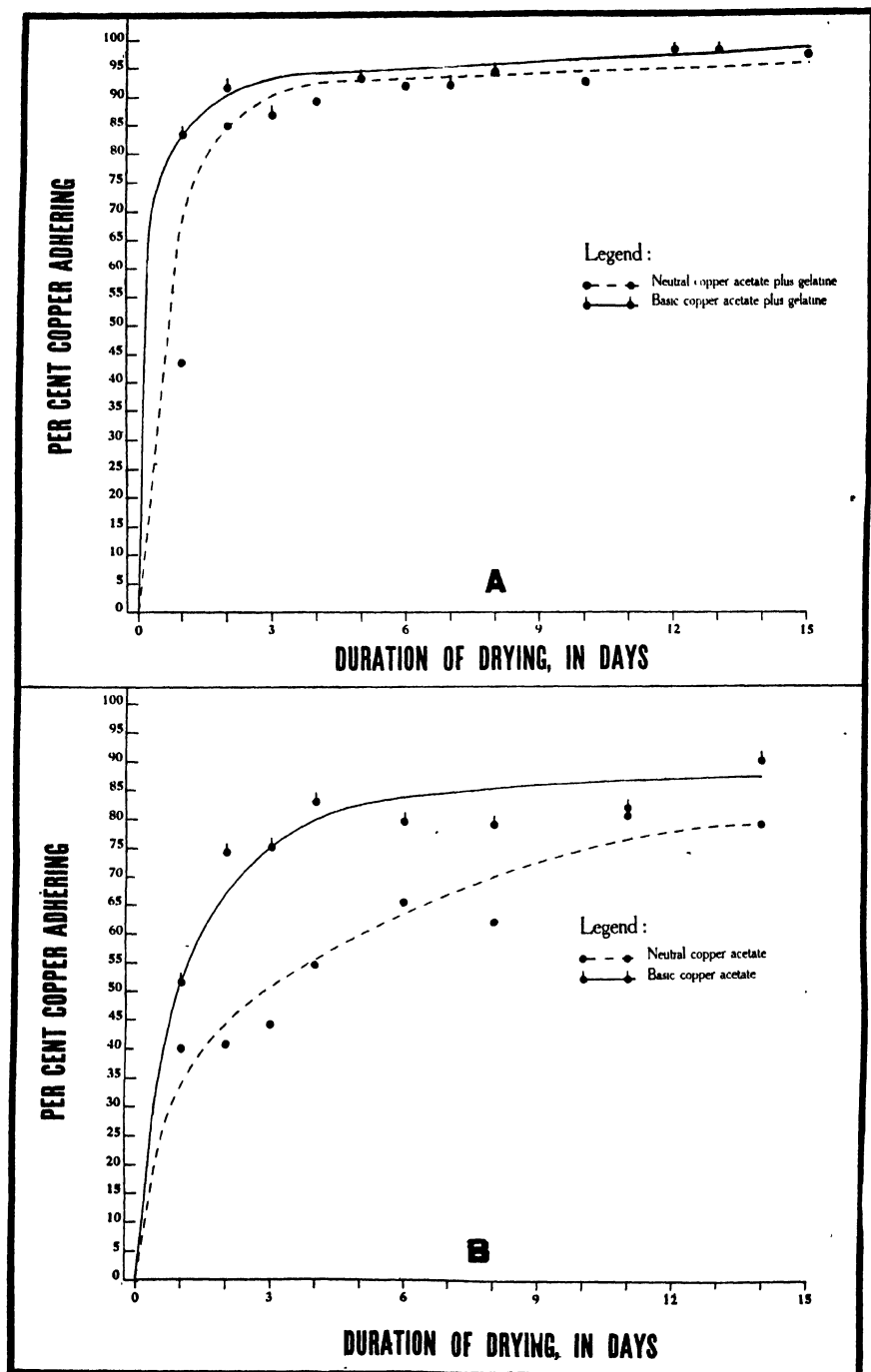


FIG. 1. Effect of length of time of drying with and without the addition of gelatine on the adhesiveness of the copper acetates.

as was first pointed out by Chuard and Porchet<sup>1</sup>, of basic copper acetate mixed with hydroxides the degree to which the decomposition occurs depending on the temperature prevailing at the time the fungicide is applied. In cold weather the decomposition occurs more slowly and is less complete than in warm weather. Basic copper acetate dissolves more or less completely in cold water forming a blue-green solution with a faint acetic odor and decomposes more readily and completely on drying than does the neutral salt.

The adhesiveness of the acetates of copper depends on the degree to which they decompose on drying and on the length of time that elapses between time of application and time of first washing rain. The basic acetate of copper is more adhesive than the neutral acetate of copper and decomposes more rapidly on exposure to air than the latter as will be seen from the data presented in table 1.

The data given in the table was obtained in the following manner:

Glass plates 5 inches square were thoroughly washed free from grease films, after which they were dried and set on a table adapted to support them at an angle of 20 degrees. The washes, made with chemically pure salts dissolved in distilled water, were sprayed on the plates by means of a hand sprayer and the spraying was continued until droplets approached the size at which they would coalesce and drain off the plates. After the plates had dried they were carefully examined for uniformity and divided into two lots of eight each. The plates in one lot were used for the determination of the total copper applied, while in the case of the plates of the other lot the copper adhering was determined after they had been exposed to artificial rain. The artificial rain used was ordinary tap water thrown from a Columbian lawn sprinkler standing on a level with the plates. The sprinkler occupied the center of a circle eight feet in diameter on the periphery of which the plates were placed facing inwards at a slight angle. The head of water used in running the sprinkler was regulated so that the water thrown fell by gravity upon the plates from a maximum height of 9 feet.

The data shows that the decomposition of the neutral acetate measured in terms of adhesiveness is roughly proportional to time while in the case of the basic acetate the rate of decomposition is extremely rapid during the first four days but continues from thence on more slowly. At no time does the adhesiveness of the neutral copper acetate equal

<sup>1</sup> Chuard, E. and F. Porchet. *Recherches sur l'adherence comparée des solutions de verdet neutre et des bouillies cupriques, employées dans la lutte contre le mildiou.* *Compt. Rend. Acad. Sci. Paris* 140: 1354-1356. 1905.

TABLE 1  
*Effect of length of time of drying on the adhesiveness of 1 per cent neutral and 1 per cent basic copper acetates*  
 A.—Neutral Copper Acetate

Interval between application of wash and incidence of rain	Duration of rainfalls	Amount of rainfall	Temperature of rain	Amount of copper sprayed on plates	Amount of copper adhering after rain
Days	Minutes	Inches	° C.	Grams	Per cent
1	30	0.37	20° C.	0.0133	39.85
2	32	0.43	24° C.	0.0123	40.65
3	31	0.37	18° C.	0.0187	44.38
4	32	0.43	22° C.	0.0141	54.61
6	35	0.41	24° C.	0.0172	65.69
8	30	0.38	23° C.	0.0167	62.28
11	30	0.40	25° C.	0.0171	80.70
14	34	0.32	18° C.	0.0187	79.14

TABLE 1 (Continued)  
B.—Basic Copper Acetate

Interval between application of wash and inci- dence of rain	Duration of rainfall	Amount of rainfall	Temperature of rain	Amount of copper sprayed on plates	Amount of copper adhering after rain	
					Grams	Per cent
Days	Minutes	Inches	° C.	Grams		
1	38	0.36	17° C.	0.0167	0.0086	51.49
2	30	0.45	23° C.	0.0194	0.0144	74.23
3	20	0.28	19° C.	0.0194	0.0146	75.26
4	31	0.48	20° C.	0.0155	0.0129	83.23
6	36	0.44	18° C.	0.0118	0.0094	79.66
8	58	0.79	18° C.	0.0135	0.0107	79.26
11	30	0.33	20° C.	0.0156	0.0128	82.05
14	39	0.55	20° C.	0.0269	0.0250	90.29



TABLE 2

*Effect of length of time of drying on the adhesiveness of 1 per cent neutral and 1 per cent basic copper acetate containing .05 per cent gelatine*

## A. Neutral Copper Acetate

Interval between application of wash and incidence of rain	Duration of rainfall	Amount of rainfall	Temperature of rain	Amount of copper sprayed on plates	Amount of copper adhering after rain	
					Grams	Per cent
Days	Minutes	Inches	° C.	Grams		
1	30	0.46	19° C.	0.0177	0.0077	43.50
2	31	0.45	20° C.	0.0237	0.0201	84.81
3	32	0.49	18° C.	0.0187	0.0170	90.91
4	30	0.38	19° C.	0.0223	0.0199	89.24
6	30	0.41	21° C.	0.0228	0.0209	91.67
8	30	0.32	19° C.	0.0225	0.0211	93.78
10	32	0.46	19° C.	0.0263	0.0243	92.39
15	30	0.45	23° C.	0.0177	0.0172	97.12

## B. Basic Copper Acetate

Interval between application of wash and incidence of rain	Duration of rainfall	Amount of rainfall	Temperature of rain	Amount of copper sprayed on plates	Amount of copper adhering after rain	
					Grams	Per cent
Days	Minutes	Inches	° C.	Grams		
1	42	0.43	17° C.	0.0156	0.0136	83.33
2	35	0.32	17° C.	0.0144	0.0129	89.58
3	30	0.40	21° C.	0.0228	0.0198	86.84
5	30	0.43	22° C.	0.0233	0.0217	93.13
7	31	0.53	22° C.	0.0162	0.0149	91.97
8	31	0.40	21° C.	0.0225	0.0210	94.27
12	30	0.46	22° C.	0.0251	0.0248	98.01
13	31	0.41	22° C.	0.0246	0.0241	97.97

that of the basic acetate of copper. The facts brought out in the table are more strikingly shown in figure 1, B. Adhesiveness is an important requisite in a fungicide and should only be sacrificed when this sacrifice is made in order to secure an important property that would not otherwise be had. In the case of the acetates of copper a sacrifice of adhesiveness is only justified if increased toxicity of the unit copper is thereby secured. In other words, in order for the neutral acetate of copper to be of commensurate value to the basic acetate of copper in practise it should be able to prevent the germination of fungous spores not affected by the latter. Obviously the spores in question will have to be unaffected by small amounts of soluble copper. The uredospores of *Puccinia antirrhini*, for example, fulfill the conditions required. When neutral and basic acetate of copper are tested following the method of Reddick and Wallace<sup>1</sup> and using the uredospores of *P. antirrhini* we have not found the neutral acetate to possess measurably greater toxicity than the basic acetate. We would be inclined, therefore, to rule neutral acetate of copper off the list of fungicides were it not for the fact that it is more readily obtained than the basic acetate and can have its adhesiveness greatly increased at small expense by the addition of 0.05 per cent. of gelatine. The use of gelatine for the purpose of increasing the adhesiveness of acid washes was first proposed by Vermorel and Dantony<sup>2</sup> and when used with the acetates of copper proves extremely beneficial. We have shown in a previous paper<sup>3</sup> that gelatine increased the adhesiveness of neutral copper acetate 14.4 per cent. and the basic acetate 12.3 per cent when the fungicides were applied to *Coleus* leaves. In figure 1, A we give the results obtained on glass plates. The data shows very clearly that the addition of 0.05 per cent. gelatine very materially increases the adhesiveness of the salts, the adhesiveness of neutral copper acetate at the end of forty-eight hours being virtually the same as that of the basic acetate and only slightly lower at each of the subsequent periods of observation. The adhesiveness of neutral copper acetate plus gelatine after standing twenty-four hours is 9.1 per cent. greater than that of the wash prepared without gelatine, but after forty-eight hours the difference in favor of the wash plus gelatine reaches the imposing figure of 108.6 per cent. In the case of the basic acetate of copper the increase in adhesiveness brought about by the addition

<sup>1</sup> Reddick, Donald and Errett Wallace. On a laboratory method of determining the fungicidal value of a spray mixture or solution. *Science* 31: 798. 1910.

<sup>2</sup> Vermorel, Victor and E. Dantony. La défense de nos jardins contre les insectes et les parasites p. 199. Villefranche, Bur. Progres Agr. and Vit. 1914.

<sup>3</sup> Loc. cit. ante. p. 441.

of gelatine is 61.8 per cent. at the end of the first day of drying and only 20.6 per cent. at the end of the second. The decomposition of the basic acetate occurs mostly during the first twenty-four hours following the application of the wash while in the neutral acetate the decomposition is greater during the second twenty-four hours. It is, of course, desirable to have a fungicide reach its maximum adhesiveness as soon after drying as possible, hence even with the addition of gelatine the neutral acetate of copper is slow in reaching the desired state and in this respect is noticeably inferior to the basic acetate. Neutral acetate of copper plus gelatine is not as adhesive as basic acetate of copper plus gelatine, the inferiority being especially marked for periods following the application of the wash of less than forty-eight hours, but as will be seen from a consideration of figure 1, is so superior to the basic acetate without the addition of gelatine that it deserves to be introduced into our formulae as a substitute for use when the latter is not obtainable.

It follows from the study we have just made of the acetates of copper that these salts are deserving of a position in our fungicide formularies. We propose, therefore, the following formulae. The weaker strength is intended for use when the acetate is to be used in lieu of a cuprammonium, the stronger when it is desirable to use a colorless wash in lieu of Bordeaux mixture and of the same fungicidal value.

Stock solutions of the copper acetates should be prepared by suspending the salts in a gunny sac or cheese cloth bag near the surface of the water following the procedure in common use when making a stock solution of copper sulphate. The water used should be cold (15° C.). No attempt should be made to hasten dissolution by means of hot water as the salts will decompose and decomposition greater than that due to the hardness of the water is to be avoided. The stock solution should be made to contain 1 pound per gallon. There will, then, be required for 50 gallons of wash.

Water	49 gallons	46 gallons
Basic acetate copper (stock solution)	1 "	4 "

When gelatine is added to increase adhesiveness always required when the neutral acetate is used, the formula becomes

Water	48 gallons	45 gallons
Stock solution	{ basic acetate      1 "      4 " neutral acetate	
Stock solution of gelatine	1 "	1 "

The stock solution of gelatine is made by dissolving 4 ounces of leaf gelatine in 5 quarts boiling water, cooling to about 40° C. then adding

4 quarts of this stock solution to the acetate previously diluted with the water and thoroughly stirring. This is most readily accomplished if the solution of gelatine is added after the water and the stock solution of acetate have been placed in the spray tank.

#### SUMMARY

1. The acetates of copper are excellent fungicides and deserve especial consideration when a colorless deposit is required.

2. The acetates of copper are less injurious to the plant sprayed than the cuprammoniums and are to be preferred to them.

3. Basic acetate of copper adheres much better than the neutral acetate of copper.

4. An addition of gelatine increases the adhesiveness of the acetates markedly, the greatest benefit occurring to the neutral acetate.

NEW HAMPSHIRE AGRICULTURAL EXPERIMENT STATION  
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# THE GROWTH OF THE WHEAT SCAB ORGANISM IN RELATION TO HYDROGEN-ION CONCENTRATION.

JEAN MACINNES

WITH ONE FIGURE IN THE TEXT

A strain of *Fusarium*, isolated from scabby wheat in Minnesota, has been found to be capable of growing in solutions varying through an

TABLE 1

*Character of growth of the wheat scab organism in relation to hydrogen ion concentration*

Expt. No. Flask No.	Buffer solution (c. c.)		Sodium hydroxide (c.c.—1N)		Distilled water (c. c.)		Czapek's solution (c. c.)		Initial pH <sup>2</sup>		Character of growth <sup>3</sup>	
	1	2	1	2	1	2	1	2	1	2	1	2
1	1	1	0	0	35	45	10	10	3.0	3.0	very slow	very slow
2	1	1	5	5	30	40	10	10	3.5	3.9	slow	slow
3	1	1	10	10	25	35	10	10	4.6	4.9	good	good
4	1	1	15	15	20	30	10	10	5.8	6.	good	good
5	1	1	20	20	15	25	10	10	7.2	7.1	good	good
6	1	1	25	25	10	20	10	10	9.0	9.8	good	good
7	1	1	30	30	5	15	10	10	10.6	?	slow	slow
8	1	1	35	35	0	10	10	10	12.0 <sup>1</sup>	11.7	very slow	very slow
9	—	1	—	40	—	5	—	10	—	11.9	—	none
10	—	1	—	45	—	0	—	10	—	12.1	—	none

<sup>1</sup> Approximate.

<sup>2</sup> Average of at least 2 determinations.

<sup>3</sup> Experiment 1 run in triplicate, Experiment 2 with 6 similar flasks for each set.

unusually wide range of hydrogen-ion concentration. The organism grew in nutrient media ranging from pH 3.0 to pH 11.7 (Tab. 1). Definite results have not yet been obtained for concentrations immediately below pH 3.0 or above 11.7, but indications are that these figures are close to the acid and alkaline limit for growth.

The organism was grown in a modified Czapek's solution<sup>1</sup> to which a mixed buffer solution was added, the pH being varied by the addition of increasing amounts of sodium hydroxide.<sup>2</sup> The buffer solution was made up as follows:

5.252 gm. citric acid  
1.876 gm glycocoll  
2.269 gm.  $\text{KH}_2\text{PO}_4$   
50 cc. distilled water

The various constituents of the medium were sterilized before mixing in order to prevent the breaking down of the sugar which takes place in the more alkaline solutions when heated.

The solutions, in 56 c. c. portions, were mixed in sterile flasks of 150 c. c. capacity and left to incubate for at least 24 hours in order to insure sterility. Flasks showing cloudiness or other contaminations were discarded. The inoculations were made with young rapidly growing mycelium of the organism taken from potato dextrose slants. Uninoculated flasks, in general, remained sterile throughout the two or three weeks during which the experiments were conducted.

The hydrogen-ion concentration of the solutions was determined at the beginning of the experiments and at various intervals thereafter by means of a Clark (4) hydrogen electrode, including the shaking cell as described by him. As growth proceeds, the hydrogen-ion concentration of the solutions is changed by the organism, those on the acid side tending to become more alkaline and those on the alkaline side more acid. Further work on the change of pH by the organism is in progress.

<sup>1</sup> Modified Czapek's solution as used:

$\text{MgSO}_4$  1.0 gm.  
KC 1.0 gm.  
 $\text{FeSO}_4$  0.02gm.  
 $\text{NaNO}_3$  4.0 gm.  
Sugar 30.0 gm.  
Distilled water 1 liter.

Sterilized at 5 lbs. pressure for one half hour.

(The phosphate was omitted due to its presence in the buffer solution).

<sup>2</sup> The sodium hydroxide stock solution was prepared in the manner suggested by Clark (4), in order to prevent the formation of carbonates by the exposure to the  $\text{CO}_2$  of the air.

It is interesting to compare the pH range of this organism with that of other micro-organisms. Hopkins (10), working with a wheat scab organism (*Gibberella saubinetii*), obtained growth in nutrient solutions varying in hydrogen-ion concentration from pH 2.5 to pH 5.5, the upper limit not being determined. Brightman, Meachem, and Acree (2) found that the organism which causes chestnut blight (*Endothia parasitica*) grew in solutions varying in hydrogen-ion concentration from pH 4.0 to pH 8.5. Wakeman (18) in determining the changes in

Organism	Hydrogen-ion concentration (pH)												Author- ity
	1	2	3	4	5	6	7	8	9	10	11	12	
FUNGI													
Fusarium sp.....													(10)
Gibberella saubinetii.....													(2)
Endothia parasitica.....													(5)
Aspergillus niger.....													(18)
Actinomyceetes.....													(15)
Wood destroying fungi...													
BACTERIA													
Bacillus radicola.....													(9)
Bacillus botulinus.....													(13)
Streptococcus lactis.....													(17)
Bacillus coli.....													(19)
Bacillus subtilis.....													(11)
Bacillus typhosus.....													(16)
Bacillus diphtheriae.....													(3)
Pneumococcus.....													(7)
Streptococcus.....													(1)
pathogenic.....													
non-pathogenic.....													

FIG. 1. The Relation of a Number of Pathogenic Micro-organisms to Hydrogen-ion Concentration.

reaction due to the growth of *Actinomyces*, found that the acid and alkaline limits for growth were between pH 5.0 and pH 8.7, varying somewhat with the species. Meacham (15) found that four common wood destroying fungi develop normally in media having a very high acidity (pH 1.7) but no data are given for the alkaline limit. *Aspergillus niger* is reported by Currie (5) to grow at pH 1.4-1.6, the critical alkaline concentration not being determined.

Much more work has been done with bacteria in connection with

tolerance to hydrogen-ion concentration, especially those bacteria causing human and animal diseases. An excellent summary of this work is given in a recent article by Dernby (6), in which he shows that none of these organisms will grow in a medium more acid than pH 4.5 or more alkaline than pH 9.0. Fred and Loomis (9), on the other hand, found that *Bacillus radicola* developed normally at all hydrogen-ion concentrations between pH 3.5 and pH 11.1. The only other reference to organisms tolerating as wide a range of hydrogen-ion concentration as that of the *Fusarium* reported in this paper is that of Itano, Neill, and Garvey (13). Some strains of *Bacillus botulinus* were found by them to grow in acid solutions of pH 4.0, while others would not grow in solutions more acid than pH 6.0. On the alkaline side, some strains would develop normally at pH 12.0 but others not above pH 9.0. According to Wyeth (19) *Bacillus coli* can grow at pH 4.27 to pH 9.87, and Itano (11) has given the range for *Bacillus subtilis* as being from pH 5.0 to pH 10.0. On the other hand, the more highly specialized Streptococci studied by Ayers, Johnson, and Davis (1) have been found extremely sensitive to slight changes in hydrogen-ion concentration and to develop normally only between pH 5.4 and 6.0.

A graphic representation of the acid and alkaline limits of a number of common bacteria and of all the fungi mentioned above is given in figure 1. It is evident that the organism used in these experiments is exceptional, particularly among the fungi, in the wide range of hydrogen-ion concentration in which it will grow. This organism is known to be capable of attacking a very large number of hosts.<sup>1</sup> If other more specialized *Fusaria* are shown to be more limited in their relations to hydrogen-ion concentration these results would seem to be of special significance.

DEPARTMENT OF BIOLOGY

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<sup>1</sup> Data by MacInnes and Fogelman to be published elsewhere.



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## SOME EXPERIMENTS WITH AZUKI-BEAN MOSAIC

TAKASHI MATSUMOTO

In a field at Morioka College of Agriculture and Dendrology a considerable percentage of a typical mosaic disease of Azuki beans, *Phaseolus radiatus*, var. *aurea*, was found in July, 1921. The disease spread very rapidly, especially in a field of the variety Kensaki so that about 70 per cent of the plants were more or less affected before harvest time. The disease is a typical mosaic and resembles that of soybean mosaic as described by Gardner and Kendrick<sup>1</sup>. The diseased plants are more or less stunted, and sometimes are accompanied by a slight distortion of young leaves. The leaflets present an unmistakable mottling which is usually more pronounced on young leaves. The dark green parts of the affected leaves are slightly raised above the surrounding surface, thus giving the so called "puffy" appearance. In most cases the dark green parts predominate, and sometimes present map-like features, being intermingled with light green parts.

*Anatomical structures of the diseased leaves.* Materials were fixed in absolute alcohol containing 25 per cent of glacial acetic acid, afterwards they were cut with a microtome and the sections were stained with Delafield's haematoxylin. The anatomy of the diseased leaves is found to be identical with that of cucumber mosaic as described by Doolittle<sup>2</sup>. The mesophyll of the dark-green parts of the diseased leaves is strikingly thicker than that of the light-green or yellowed parts (Figs. 1, 2). The palisade cells of the former are markedly longer and narrower than those of the normal tissues. The corresponding cells of the light-green parts are much shorter than those of the normal ones, more or less isodiametric, and show a tendency to crowd together so closely that their intercellular spaces are almost invisible. The second important difference between these two different portions occurs in the spongy parenchyma. The spongy parenchyma of the light-green parts is also strikingly compact as compared with that of the dark-green areas. The chloroplasts of the former, as in cucumber mosaic, are slightly smaller and less in number than in the darker areas.

*Starch and sugar in mosaic area.* Starch tests were made after the

<sup>1</sup> Gardner, Max W. and James B. Kendrick. Soybean mosaic. Jour. Agric. Res. **22**: 111-113. 1921.

<sup>2</sup> Doolittle, S. P. The mosaic disease of cucurbits. U. S. Dept. Agric. Bull. 879. 69 p. 1920.

method described by Freiberg<sup>1</sup>. Sections of the diseased leaves were mounted in water, and the slide drawn through the flame of a micro-burner until the drop of water began to simmer. A drop of 75 per cent.

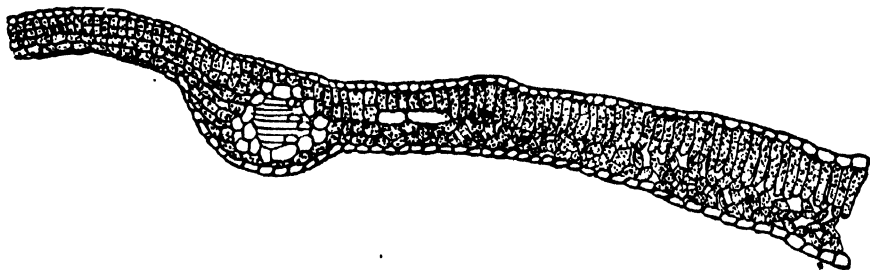


FIG. 1. THE MESOPHYLL OF MOSAIC AZUKI BEANS. RIGHT, DARK-GREEN PORTION; LEFT, LIGHT-GREEN OR YELLOWED PORTION

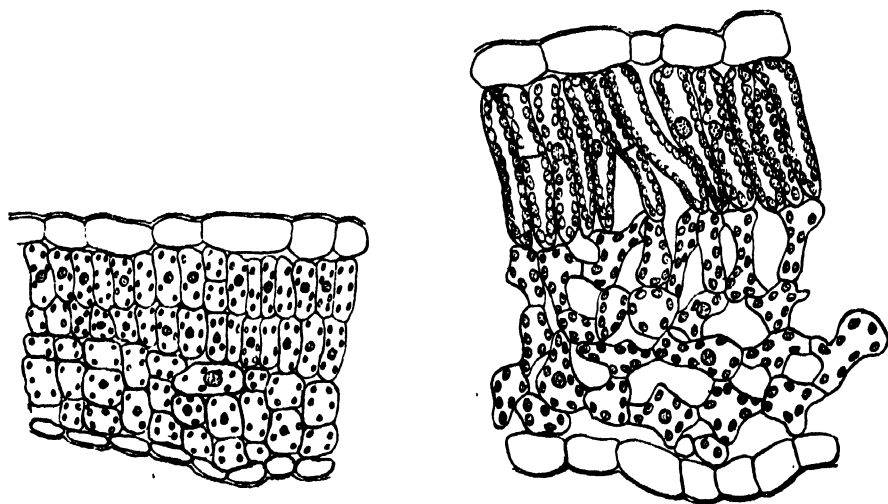


FIG. 2. THE MESOPHYLL OF MOSAIC AZUKI BEANS. RIGHT, DARK-GREEN PORTION; LEFT, LIGHT-GREEN OR YELLOWED PORTION.

alcohol and a drop of standard iodine were then added, after which the sections were examined under a microscope. In this simple test it was readily determined that starch in the dark portions is more abundant than in the light-green parts. Woods<sup>2</sup> found that the light-green parts

<sup>1</sup> Freiberg, Geo. W. Studies in the mosaic disease of plants. *Ann. Missouri Bot. Garden* 4: 175-232. 1917.

<sup>2</sup> Woods, Albert F. U. S. Dept., Agric., Bur. Pl. Ind. Bull. 18. 24 p. 1902.

of the mosaic leaves of tobacco picked early in the morning had a higher starch content than the dark-green areas, as indicated by iodine tests, and attributed this fact to an inhibitory action exerted on diastase by oxidase. The observations of the writer, however, accord with those of Freiberg and lead to the conclusion that starch is more abundant in the dark parts, regardless of the time of day. This evidence is more clearly and decidedly assured by comparing a mosaic leaf treated with iodine solution with a picture of the same leaf which has been photographed just before a treatment with alcohol for extracting chlorophyll.

Attempts were made to determine quantitatively the amount of sugar in the two portions by using the well-known Fehling's solution. For this particular study this method was not very satisfactory, owing to the great diffusibility of the sugar in the tissues of the leaves. Nevertheless from careful repetitions it is concluded that sugar is more abundant in the dark-green parts than in the light-green parts.

*Varietal susceptibility.* No extensive tests on varietal susceptibility have been made but some attempts have been made in a limited area of a test garden. In this connection thanks are due to Mr. Kenkichi Sato, a colleague.

The following 23 varieties were under observation. Of 25 plants of each variety examined the percentage of mosaic was found to vary from no infection at all to 100 per cent. infection. The percentage infection in each variety is indicated by the numeral following the name of the variety. Aka-azuki, 4; Benizu, 0; Bon-Azuki, 24; Dainagon, 8; Higashinimen, 12; Chin-yen, 0; Kensaki, more or less in all plants; Koshiroazuki, 16; Kwa-gin, 16; Maruba, 4; Min-zan, 0; Vuminza, 4; Mumeimadara, 4; Muroran, 4; Okute-azuki, 8; Ri-don-yo, 0; Ri-kwan-don, 0; Rin-san-in, 0; Ryokuzu, 0; Shiboriwake, 8; Shiro-azuki, 8; Shirosaya, 4; Wasedainagon, 0;

As will be seen, some varieties were free from the disease. It remains to be determined, however, whether the varieties are entirely immune or not. Attempts to communicate the disease to related species and to cucurbits are in progress.

MORIOKA, JAPAN.

# POTENTIAL SPORIDIA PRODUCTION PER UNIT IN CRONARTIUM RIBICOLA

MINNIE W. TAYLOR

WITH ONE FIGURE IN THE TEXT

Actual counts of telial columns were made on 68 leaves of 12 species of *Ribes*—*R. americanum*, *R. cynosbati*, *R. glandulosum*, *R. lacustre*, *R. nigrum*, *R. odoratum*, *R. oxyacanthoides*, *R. rotundifolium*, *R. setosum*, *R. triste* and *R. vulgare*—taken from the field collections in the Office of Forest Pathology of the United States Department of Agriculture at Washington, D. C. These leaves were selected on the basis of the average size and the maximum infection represented in the collections. The writer is indebted to Dr. Perley Spaulding and to Dr. R. H. Colley for valuable advice and assistance.

The average number of teliospores in a single telial column was estimated by counting the spores in a series of cross and longitudinal sections of telial columns from *R. nigrum*. Each spore was considered as having a potential capacity for producing 4 sporidia. The number of spores was estimated to be 1500 with 6000 potential sporidia per column. These constants were used in estimating the spores and potential sporidia in the columns from all the species studied with the exception of those from *R. glandulosum*. The columns of this latter species were conspicuously longer and narrower than those of the *R. nigrum* type. Therefore, separate counts were made and the estimates of 936 spores with 3744 potential sporidia per column were obtained.

The area of all leaves of each species was determined in square inches by planimeter measurement. Actual count was made of every telial column on every leaf of each species examined and the number of teliospores estimated. The average number of teliospores per square inch per species was obtained by dividing the total count per species by the total leaf area per species. The quotient multiplied by 4 gave the average number of potential sporidia per square inch of leaf surface. This last figure was used as the unit for comparison.

The average number of potential sporidia per square inch of leaf surface for each of the 12 species of *Ribes* studied is shown below:

<i>Ribes lacustre</i>	281,280
“ <i>triste</i>	360,000
“ <i>setosum</i>	756,540
“ <i>americanum</i>	974,280
“ <i>cynosbati</i>	1,141,920

<i>Ribes vulgare</i>	1,305,000
" <i>glandulosum</i>	1,679,484
" <i>rotundifolium</i>	2,746,140
" <i>oxyacanthoides</i>	3,706,020
" <i>odoratum</i>	5,480,640
" <i>aureum</i>	5,645,520
" <i>nigrum</i>	16,799,400

As previously stated, the above figures are based on actual counts of telial columns on representative *Ribes* leaves in the herbarium collections. Since these collections were not made with this study in view and do not always show average field conditions, it is obvious that the figures cannot be applied rigidly in estimating the relative potential danger of any given species of *Ribes*.

Field notes indicate that *R. americanum* is seldom found infected unless it happens to be in the immediate vicinity of diseased pines, in which case the infection may be quite heavy. Moreover, under local conditions, *R. vulgare* may occasionally be found heavily infected. In the middlewest, *R. cynosbati* seems to be more susceptible than *R. glandulosum*.

In order to arrive at definite constant figures, it would be necessary to make a study of material collected for this particular purpose from average bushes of each species, taking into account the various localities in which the species occur, the average size of bush for each species, the average size of leaf for each species, the average susceptibility of the species and the average amount of infection per bush per species. This would be a difficult task and until such an ideal collection of material is available, the information obtained from these herbarium collections can be used as a basis for a general estimate of the potential sporidia production for the species studied.

A subsequent study, based upon material actually collected for the purpose, will doubtless show certain changes in the relative positions of the species but the indications are that the cultivated *R. nigrum* will still head the list in potential sporidia production per square inch of leaf surface and when present must be regarded as the most dangerous *Ribes* neighbor of the white pine.

OFFICE OF FOREST PATHOLOGY

BUREAU OF PLANT INDUSTRY

UNITED STATES DEPARTMENT OF AGRICULTURE

WASHINGTON, D. C.

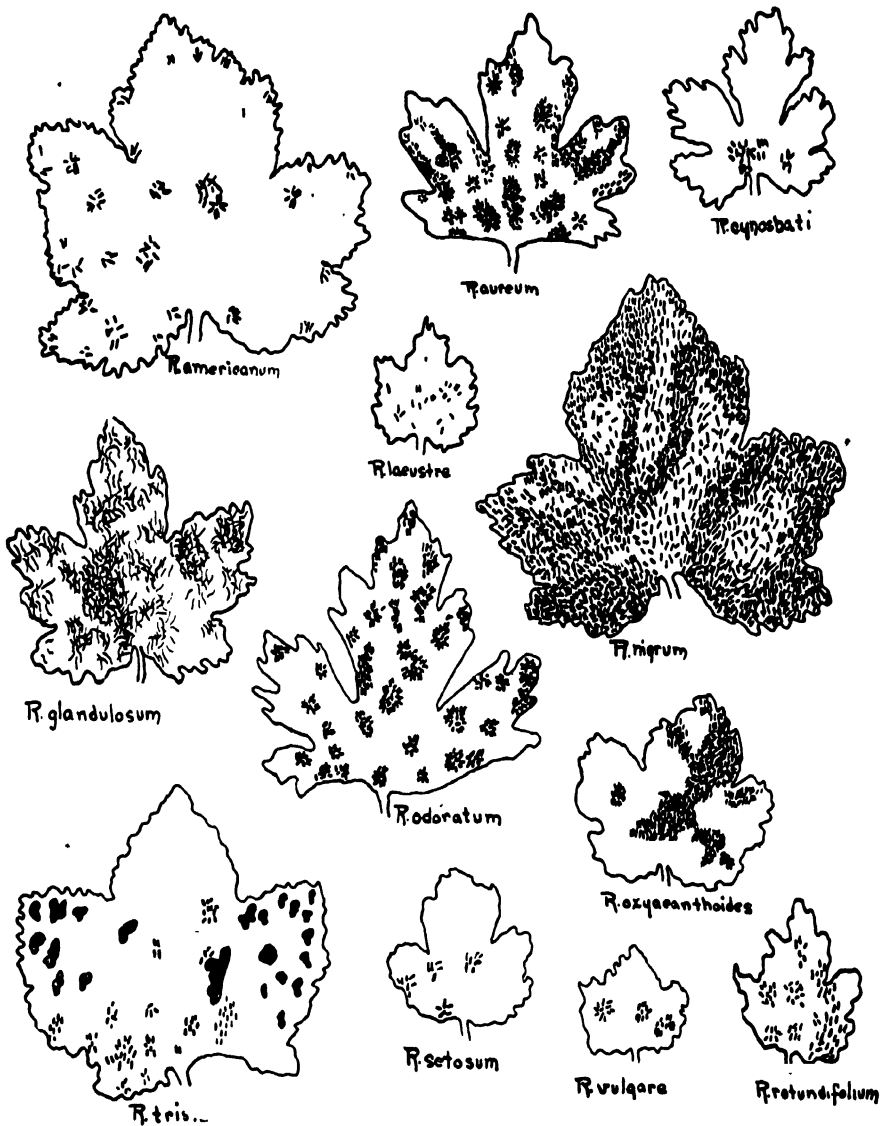


Fig. 1—Diagrammatic drawings to show average shape and size and relative amount of infection of the twelve species of *Ribes* leaves studied.  $\frac{1}{2}$  natural size.

Note—The leaves of *R. triste* contained many holes—due probably to a “shot hole” fungus—which were not taken into consideration in computing the area.

## PHYTOPATHOLOGICAL NOTES

*Relation of plant pathology to the newly organized Science Service.* No argument is needed to convince plant pathologists of the desirability of greater publicity for results of research. It is fully evident that a more complete knowledge of plant pathology and its accomplishments on the part of the general public will be helpful not only to the public itself, but also to the science through increased encouragement and support for our projects. Various individuals, both scientific and non-technical, have written popular articles on subjects pertaining to plant pathology. The merit of these articles has varied widely, and the total number appearing in the newspapers or in publications outside of experiment station literature, is small. Most plant pathologists are engrossed in their work and few have had experience in writing for the popular reader. Hence, this type of composition has been neglected and plant pathology has suffered through lack of understanding and appreciation on the part of the public.

In the recently organized Science Service, a new channel has been opened for bringing the results of scientific investigation before the public in a quick, accurate, and comprehensible way. This service, which has recently been described in *Science*,<sup>1</sup> has been made possible through the generosity of Mr. E. W. Scripps of California and is organized with headquarters in the National Research Council building at Washington. It is governed by a board of fifteen trustees of whom ten are scientists, and five are journalists. Dr. Edwin E. Slosson, who for twelve years was Professor of Chemistry in the University of Wyoming and for seventeen years was editor of *The Independent* of New York, has been selected editor. The corporation is non-profit making, all receipts from sale of articles, books, films, etc. being used to develop the service.

The object of Science Service may be given in the words of Doctor Slosson:<sup>1</sup>

"Science Service has been founded for the purpose of reaching through the press and motion pictures a wider public than is now interested in the progress of science. The only way to eliminate fake science stories that too often appear in our papers is to crowd them out with articles that are more accurate and equally interesting. . . . Science Service will spare no pains or expense in the effort (1) to give the best possible quality of reading, and (2) to give it to the largest possible number of readers."

<sup>1</sup>Slosson, Edwin E. A new agency for the popularization of science. *Science* n. s. 53: 321-322. Feb. 8, 1921.



At present Science Service is selling to a large number of newspapers a weekly news bulletin, the items in which range in length from a single sentence to 150 or 300 words. Writers of these articles are paid one cent a word. Longer articles of from 500 to 1500 words are bought by Science Service at two cents a word and used in newspapers and magazines.

This outlet for popular articles on plant pathology presents an excellent opportunity to us to get our information before the people in an easy, quick, and effective way, and we should by all means take full advantage of it. Plant Pathology, as always, should be at the front in movements of this kind. Persons interested should write immediately to Doctor Slosson and ask him for further directions as to what kind of articles he wants and how they should be written. If it is not possible for the contributor himself to write his article in finished form for publication, data should be submitted to be worked up by the editorial staff of Science Service. Lack of time or skill in writing popular articles need deter no one from doing his share in properly presenting plant pathology to the general public.

It is preferred that contributors send their papers directly to the Editor of Science Service, 1701 Massachusetts Ave., Washington, D. C., but the writer who is interested in the project as a part of the program of the Advisory Board will be glad to act as an intermediary between the members of the American Phytopathological Society and Science Service in so far as may be desired.

R. J. HASKELL, Secretary,

Advisory Board of American Plant Pathologists.

*The occurrence of Cronartium ribicola in Europe.*—The aecidial stage of *C. ribicola* chiefly occurs in Europe on the Weymouth pine (*P. strobus*), although *P. lambertiana* and *P. flexilis* may also be infected, as reported lately by Moir<sup>1</sup>. The rust is recorded by Grove as distributed over Europe, north of the Alps, it is not listed by Fragoso among fungi of the Peninsula, and we never observed it in southern or central France. In French forests, Weymouth pines occur chiefly in the Vosges; they were planted extensively in Sphagnum moors covering 800 hectares around Epinal. Cases of infection by *C. ribicola*<sup>2</sup> are recorded by Forester M. Mangin, chiefly on the border of the forests.

<sup>1</sup> Moir, W. Stuart. [Sur l' extension du *Cronartium ribicola* Dietel]. (Abstract of letter.) In Bul. Soc. Path. Veg. France, t. 7, fasc. 4, p. 100. 1920.

<sup>2</sup> Hariot, P. Sur quelques rouilles des plantes cultivées. (Abstract.) In Bul. Soc. Path. Veg. France, t. 1, fasc. 1, p. 15-16. French abstract in Min. Agr. [France], Ann. Serv. Epiphyties, t. 3, 1914. p. 22-23. 1916.

Well grown trees, averaging 0.20 m. in diameter at base may be infected to the top, as well as seedlings in nurseries. Once initiated, infection spreads rapidly; visiting a place where one of the pines was rusted in a private forest, Forester Mangin truly prophesied that all of them would die within two years. In governmental forests, however, Mangin does not consider the rust a menace, or even a serious trouble, as simple sanitary measures always restrict the infection to a few single trees; infected pines are felled and burned as soon as located, while the brushwood in the vicinity is destroyed, by assartage, to get rid of the telial hosts. A specimen of infected trunk was deposited by Mangin at the Laboratoire de Cryptogamie du Museum d'Histoire Naturelle de Paris, Specimens of *Peridermium strobi* were forwarded to the Station de Pathologie Végétale but rarely, as the disease is causing but little trouble in France; aeciospores from a *Peridermium* obtained in the Vosges, though having been travelling for a week, developed uredo sori of *Cronartium* on *Ribes uva-crispa*, within ten days of inoculation. *Peridermium strobi* was also reported from the forest of St. Amarin, Alsace.

The telial stage is not common; we never found it on wild *Ribes*, but it rusted cultivated *Ribes*, and more severely, *R. nigrum* of the Anjou, in 1917<sup>1</sup>.

We are indebted to Fischer<sup>2</sup> for important data about the European distribution of *Cronartium ribicola*. He first reported the rust on *Ribes petracum* in the Engadine (1895), where Schellenberg found the aecial stage in 1903 on *Pinus cembra*. From the Engadine, where Fischer considers the rust indigenous, it never spread to the rest of Switzerland. However, infected seedlings of *P. strobus* imported from Germany, started a rust epidemic from the Locle, in the year 1904-05; this epidemic spread rapidly all over Switzerland. Rust damage was complained of in Boudry (Neufchatel, 1909), in Montagny, and in Bern (1911), and even to Chateau d'Oex (1915). In the district of Zurich, where the disease is most severe, the Weymouth pine is planted no more. Curiously enough, this epidemic was demonstrated by Fischer actually to be of foreign origin, having originated in Siberia, and spread to Switzerland through Germany, whereas *Cronartium ribicola* from the Engadine remained restricted to that district.

*Ribes nigrum* and *R. aureum* are most severely rusted, and *R. sanguineum*

<sup>1</sup> Marchal, Paul, and Arnaud, G. Rapport phytopathologique pour les années 1916 et 1917. Groseilliers. In Min. Agr. [France], Ann. Serv. Epiphyties, t. 5, 1916-17, p. 29. 1918.

<sup>2</sup> Fischer, Ed. Pilze. In Ber. Schweiz. Bot. Ges., Heft 24-25, p. 72. 1915-16.

somewhat less so. Weymouth pines are chiefly rusted when 15 to 50 years old; completely girdled shoots are sure to die quickly; partially infected shoots may linger for months, or perhaps recover, if diseased parts be carefully excised. Good results were reported from the Belgian Arboretum of Groenendael, due to the following treatment: the rusted cortex was removed, and the wound was washed with a 10 per cent solution of potassium permanganate. To restrict the spread of *C. ribiculom*, Badoux<sup>1</sup> writes as follows:

In nursery, use only healthy, clean, and superficially disinfected seeds.

Plant healthy seedlings, obtained from uninfected nurseries.

Do not grow Ribes in the vicinity of Weymouth pine nurseries.

Do not grow Weymouth pine in infected nurseries, for some years at least.

Plant Weymouth pines among other trees.

JEAN DUFRENOY, STATION DE PATHOLOGIE VEGETALE,  
PARIS.

<sup>1</sup> Badoux, H. Le pin Weymouth (*Pinus strobus*) en Suisse. In Jour. Forest. Suisse, Ann. 71, p. 221-227, 1920; Ann. 72, p. 86-89, p. 131-135, p. 148-152, p. 165-173, 1921.

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# PHYTOPATHOLOGY

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## THE RELATION OF THE WATER PORES AND STOMATA OF THE POTATO LEAF TO THE EARLY STAGES AND ADVANCE OF TIPBURN.

B. F. LUTMAN

WITH FIFTEEN FIGURES IN THE TEXT.

The presence of hydathodes or water pores in the leaf of the potato plant seems to have been known for some time. They are mentioned incidentally by Jost (11, p. 57), in the following words: "Well known examples of the excretion of drops are furnished by the leaf apices of grasses, the leaf teeth of Fuchsia, Alchemilla, Brassica, and the potato." Clinton (3, p. 742-743) in discussing the advantages derived from spraying with Bordeaux mixture in years in which no blight appeared, suggested "that the results are largely due to the conservation of moisture in the leaves in dry seasons by clogging up the stomata and water pores with the sediment of the spray. The reasons for this belief are: (1) that the potato leaves, through their numerous stomata and terminal water pores, lose water very easily and are especially susceptible to what is known as tip burn in dry seasons; (2) that the unsprayed vines uniformly suffered earlier and more severely from tip burn than the sprayed, which were green for about two weeks after the unsprayed were dead; (3) That in 1910, which was a season like the preceding years, except with a little injury from blight at the very end of the season, spraying with Sulphocide and commercial lime-sulphur, sprays with comparatively little sediment, did not prolong the life of the vines or give increased yield, while spraying with Bordeaux mixture did."

It will be noted that the occurrence of water pores at the tips of the leaflets is merely mentioned by Jost, and by Clinton. Spanjer (22), in his list of plants known to possess water pores did not include the potato nor, in fact, any of the Solanaceae. Haberlandt (7, 9), in his extensive contributions to the comparative morphology of this type of plant excretory organs, did not refer to this group; neither does he mention

the potato in his long review of all the important literature in his "Physiological Plant Anatomy." Minden (14), in his list of plants known to possess some sort of an apparatus to secrete water, did not include the potato but did mention the following Solanaceae: *Solanum nigrum*, *Solanum dulcamara*, *Nicandra physaloides*, *Capsicum annuum*, *Datura laevis*, *D. stramonium*.

It would seem, therefore, that while it was commonly known that the potato plant was able under the proper conditions to exude water in the form of drops through some sort of water pores, no accurate study had been made of their form of arrangement. Indeed it is a moot point whether anyone heretofore has taken the trouble critically to examine the potato leaf in this respect. The attention of the writer was first called to them by the study of the venation of the leaf in its relation to the disease known as tipburn when apparently healthy leaves being prepared for the examination of the vascular system exhibited brown areas at their tips and sides in the center of which occurred the water pores.

Before describing these organs and the arrangement of the veins on the potato leaf it seems advisable to review the descriptions of similar apparatus on other plants. It should be understood at the outset, however, that many points in the physiology of this water secreting apparatus are obscure and that the principles upon which they work are more or less a matter of conjecture and controversy.

Plant physiologists in general seem to agree touching the ecological uses of these organs better than they used to do concerning the methods by which those results are obtained. Practically all plants possessing water secretory organs are shade or semishade plants in their original habitat. Most of them grow in the tropics or subtropics. Because of the very heavy rains which occur in these regions, the plants at times are compelled to throw off the superabundance of water that has been brought up from the roots into the leaves, into an atmosphere already almost saturated with water vapor. Transpiration under such circumstances is necessarily small, the output is less than intake and a very high turgor exists throughout the plant, especially in the vessels which conduct the water. The only possible means of pressure release is the discharge of the water into the intercellular spaces of the leaves. However, such flooding of the air chambers leads to a decidedly lessened exchange of gases and to curtailed starch assimilation. Furthermore, it has been suggested that retardation of transpiration and evaporation tends to slow down the upward current of soil water into the plant, to a considerable extent cut off the supply and, consequently, to lessen the crop.

The agreement is not so general among plant physiologists in regard

to the physical agencies which bring about the extrusion of the water. Root pressure or bleeding pressure must furnish most of the force back of the passage of water through the walls of the tracheids, but Haberlandt is inclined to assign an important rôle to the epitheme cells which surround the vascular bundles of many varieties of plants, especially tropical species. This tissue serves not as a sieve, as might be the case if the root pressure was the sole force operative, but plays an active part in the control of the flow of the water which passes through it. The water must pass through these cells in order to enter the intercellular spaces and from them to flow into the water cavity under the guard cells and it is the function of these epitheme cells to maintain these intercellular spaces and cavity full of water. The large nucleus of the epitheme cells and their abundant supply of plasma is similar to that of the gland cells. Certain experiments of Haberlandt's seem to corroborate this conception of the function of the epitheme as a tissue.

The ability of the guard cells to open and close under different atmospheric conditions is doubtless a factor, but there seems to be some diversity in their mode of operation among such plants as *Secale cereale*, *Triticum vulgare* Vill., *Zea mays* L., etc.

The substitution of artificial pressure for root pressure was developed by de Bary (2) and Moll (15) but has been most extensively used by Haberlandt and Spanjer (22, p. 75). The method generally used was as follows: A branch of the plant was cut off and attached to the short arm of a J-tube and water or other liquids were forced into it by means of a mercury column 10 to 40 cm. in length, located in the longer arm, the plant in the meantime being kept under a bell jar lined with damp filter paper in order to curtail respiration as much as possible. Spanjer was able in this way to force water through the leaves in  $2\frac{1}{2}$  to 3 hours, but cosin solution and a 2.5 per cent solution of copper sulphate were 24 hours showing color in the drops.

The hydathodes were first noticed by the writer on potato leaves which he had prepared in order to examine the finer details of the vascular system, according to the method recommended by de Vries (25, p. 604-618). Other methods of rendering the leaf transparent and free from chlorophyll would probably give as good results but the following procedures will be found to be very successful with the potato leaf as, indeed, they have with most of the plants studied.

Boil the leaves for a few minutes in water, drain, soak for 12 to 24 hours in 95 per cent alcohol and then in a 5 per cent solution of sodium hydroxid for about the same length of time, wash in water for a few minutes, neutralize with a few drops of hydrochloric acid, place in 50

per cent glycerin for 24 hours, and then mount on a glass plate in pure glycerin. Small discarded photographic plates that have been cleaned are excellent for this purpose. Even very thick leaves are rendered so transparent that the entire leaf structure can be made out in optical

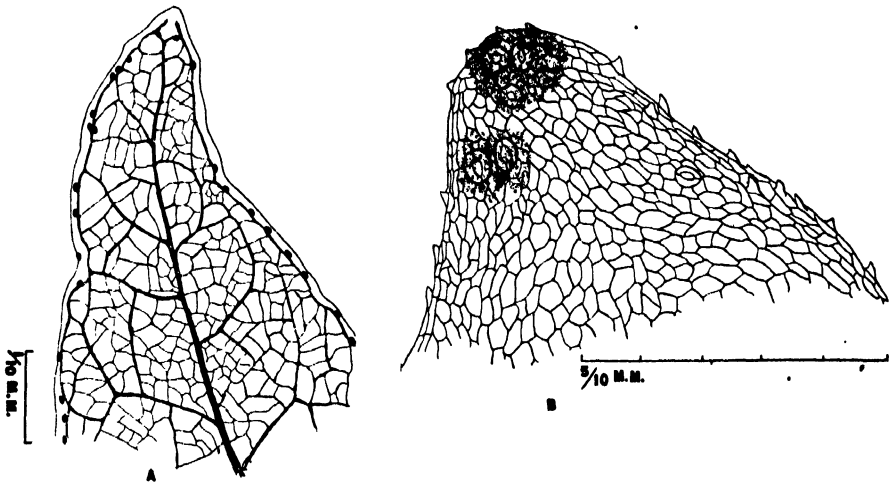


FIG. 1. A. Tip of very young leaflet showing venation and the position of the hydathodes. B. Tip of mature leaflet in surface view, showing groups of hydathodes. Dead tissue under the pores is indicated by dotting.

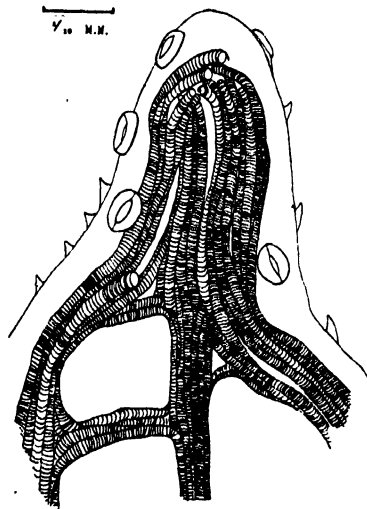


FIG. 2. Tip of leaflet, showing hydathodes and plexus of veins.

sections. An objective with long working distance is advisable, for example a Zeiss 16 mm. Apochromat with a No. 12 compensating ocular, or a Leitz  $\frac{1}{4}$  inch objective combined with a No. 3 or 4 ocular

if the leaves were covered with a large ordinary cover glass. The venation as thus shown presents no marked peculiarities except in the regular occurrence of a very highly developed marginal vein. Artschwager (1) has made a very careful study of the anatomy of the potato plant with special reference to the structure and arrangement of the vascular elements, but the marginal vein of the leaflets does not seem to have attracted his attention. A number of plants were examined to determine whether this vein is at all common, but aside from the potato the nasturtium alone showed this peculiarity. The lateral veins branch away from the midrib to form numerous anastomoses throughout the leaf, but particularly near its margin the anastomoses form an almost continuous vein. This vein disappears occasionally in folds of the leaf,

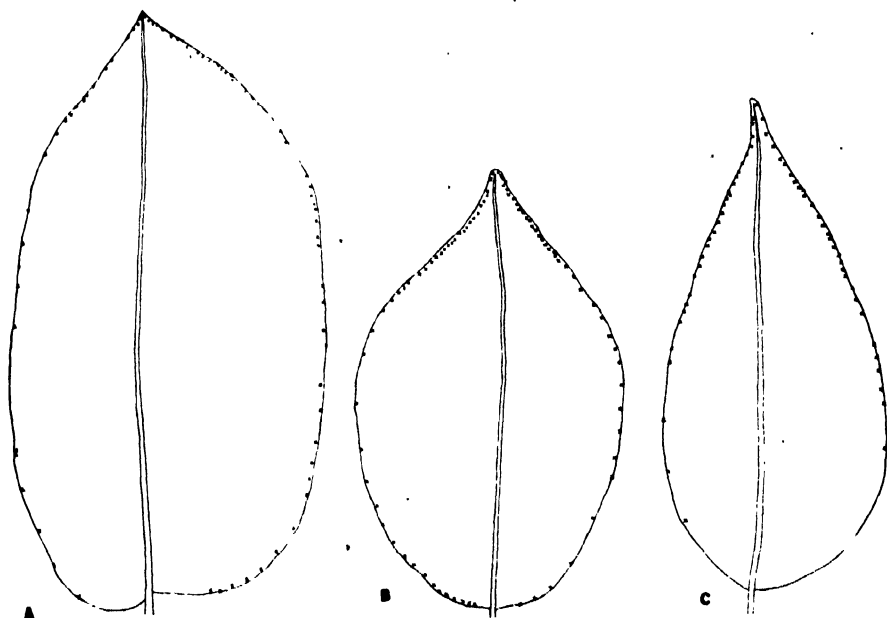


FIG. 3. Tip leaflets with position of hydathodes indicated. A and B are mature, C is a young leaflet.

but it is usually continuous for at least 95 per cent of the leaf margin.

It is composed of a varying number of tracheids and sieve tubes, the former sometimes being limited to a single large vessel, while in other places five or six tracheids may be observed. The development of this marginal vein is especially noticeable toward the tip of the leaf where it forms very important and large anastomoses with the vessels of the midrib (Figs 1, 2). In the extreme tip, (Fig. 2), the midrib and the marginal vein practically fuse to form a tangled mass of vessels,



many of which end blindly in the leaf parenchyma or under the water pores. On the basal portion of the leaflet, the marginal vein does not fuse with the midrib but runs parallel to it along the petiole, supplying the decurrent portion of the leaflet with vessels and sieve tubes. It is small in this region but it continues along this decurrent portion of the leaflet, joining by occasional small anastomoses with the midrib but continuing until it reaches the petiole of the succeeding leaflet where it forms the marginal vein. It will thus be seen that the marginal veins of all the leaflets are connected, forming a continuous series of tubes around the entire leaf.

Leaves taken from the field and examined in the manner described above, in June, 1921, showed brown areas over this marginal vein and

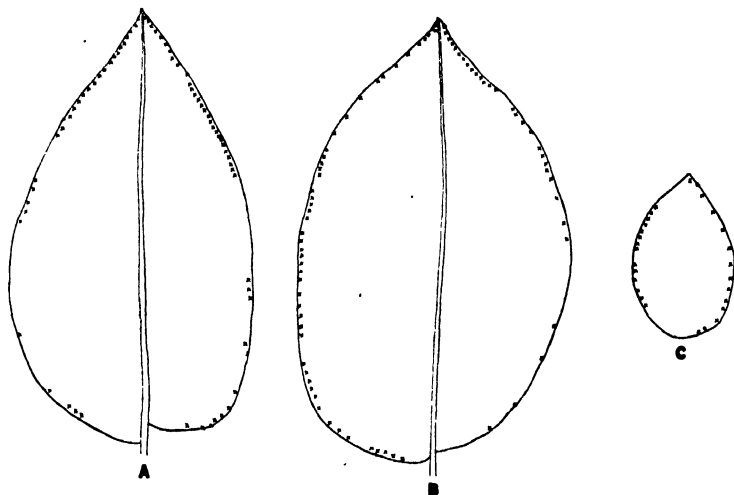


FIG. 4. A and B, side leaflets; C, a small intermediary leaflet. Hydatodes are indicated.

also at the extreme tip of the leaflet, whereas this condition had not been observed on those taken earlier in the spring from the greenhouse, which were apparently healthy and showed no signs of tipburn. A closer examination of these brown areas showed that a water pore much resembling the ordinary stoma was located above each one of these regions and was apparently the focus of the disturbance. All of these water pores were located on the upper margin of the leaf or opened out directly on the edge, but they always were in connection with the marginal vein. Whenever the marginal vein was suppressed, the water pores were also absent. A group of these organs was always clustered at the extreme tip of each leaflet on the upper side (Figs. 1, 2).

After these organs were observed on these older leaflets, many leaflets

of all ages and from different portions of the leaf were carefully studied under the microscope in order to determine their number and distribution (Figs. 3, 4). It was found that they occurred on all the margins of the leaflets but that this distribution was somewhat irregular. The greater number per unit of leaf margin occurred toward the tip, but they were present in even the basal lobes. A small number exist also on the very small leaflets located between the larger ones (Fig. 4, C). They average about 80 per leaflet, but one was found with 140. The number did not seem to vary much among the various leaflets although naturally the terminal leaflet, being usually a little larger, is likely to have more than the others.

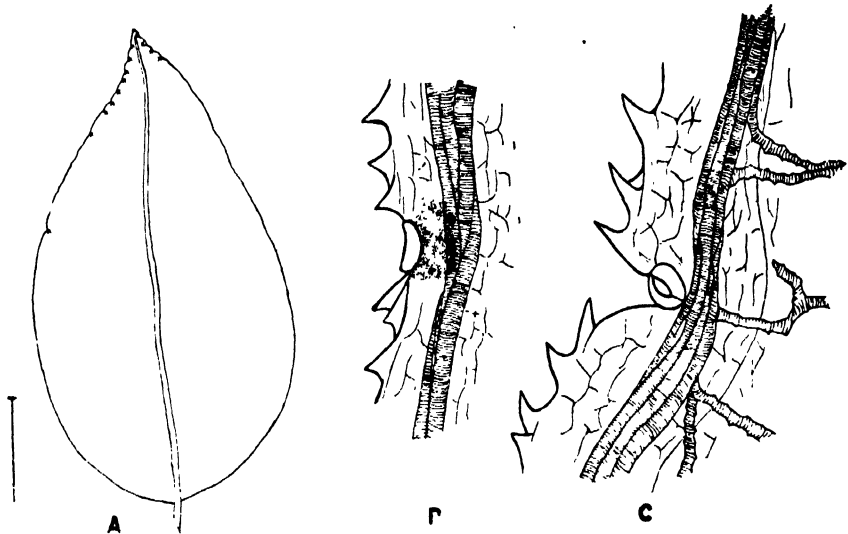


FIG. 5. A. Very young tip leaflet, true size indicated by line at the left: B and C are hydathodes in surface and partial sectional view.

Miss Rea (20) has found the hydathodes in *Campanula rotundifolia* more numerous and with larger pores on the shade shoots than on the sun shoots, as would be expected if these organs are to prevent the flooding of the internal tissues, since the danger is greater in such an environment where the transpiration is small. No attempt was made to compare the number on shade and sun potato leaves, but it is entirely possible that the high number was on a shaded leaflet.

The position of the hydathodes on the young leaflets differs somewhat from that on the older ones (Fig. 5). The young leaflets show a number of small infoldings of the margin, and it is in these curves that the hydathodes are to be found. At times the guard cells occur at the same

level as the surface, but more often they are located at the bottom of a little well formed by the margin of the leaflet (Fig. 4, C). They are much fewer in number than on the older leaflets and are limited to the tip region. The distribution of the hydathodes on a leaf about 2 cm. in length on the tip portion as shown in figure 1, A. The distribution on a leaflet of this length is very similar to that on a mature leaflet, and the small folds in the margin fill out so that the edge of the leaf is almost smooth.

The structure of the hydathodes as in sections (Fig. 6) is not unlike that of one of the stomata, but the water cavity is larger and opens directly on the vessels of the marginal vein as the epitheme, if such can be said to exist, is limited to a few scattered cells in no way very different from those of the immediately adjacent tissue. In stained sec-

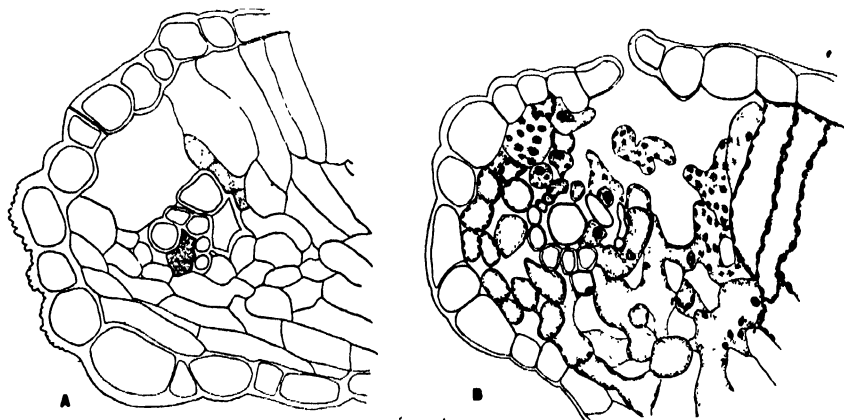


Fig. 6. A Hydathode from a section cut from fresh material;  
B, Hydathode from fixed and stained material.

tions (Fig. 6, B) the nuclei in the cells surrounding the vessels are unusually large and the cells are rich in cytoplasm, but there is no evidence that they are to be regarded as gland cells.

The relation of the hydathodes to the ordinary air stomata is an interesting one and is a part of the problem that has not been thoroughly worked out. It seems to have been avoided by the physiological morphologists who have studied these organs. Minden (14) followed the development of the water pores to some extent on the seed leaves of *Phacelia tanacetifolia*, *Solanum nigrum*, *S. dulcamara* and *Nicandra physaloides*. They arise in about the same manner as do the stomata of the Solanaceae as described earlier by Strasburger (24, p. 323) from epidermal cells which divide once to form the two guard cells. These mother cells are somewhat sunken below the level of the epidermis.

Minden found that more water pores were developed on the leaves of plants in damp situations than on these from drier locations.

In species like the potato the hydathodes with their water pores seem to be a development of the ordinary stomata (Fig. 7). The guard cells and the opening of the water pores of the potato are larger but structurally the two are identical and all intermediate types may be found on the young leaflets. No attempt has been made to follow the development of the internal structure of these hydathodes in their development, but the external appearance of the stomata and hydathodes is essentially the same. The statement that the water pores lose their ability to close is incorrect so far as the potato guard cells are concerned. Stomata

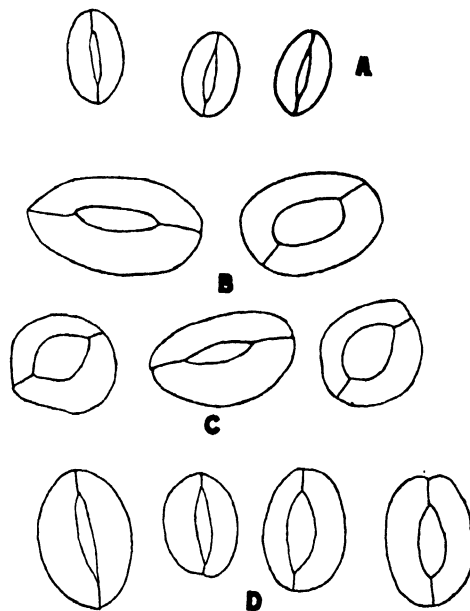


FIG. 7. A, Ordinary stomata, x300: B, C, and D, guard cells from hydathodes from three mature leaves.

from a practically full grown leaflet closed almost completely in a solution of 5 per cent glycerin, and the guard cells of the water pores behaved in a similar fashion. The guard cells do not seem to be involved in the browning of the tissue underneath the water pore in some cases, but in others these cells had suffered in a similar manner. The latter condition is to be expected if the injury is very severe.

The death of the tissues under the water pore begins at the tip end of the plant and proceeds backward under the pores on the sides of the leaflet. The pores along the basal margins are last to become involved if they ever are affected.

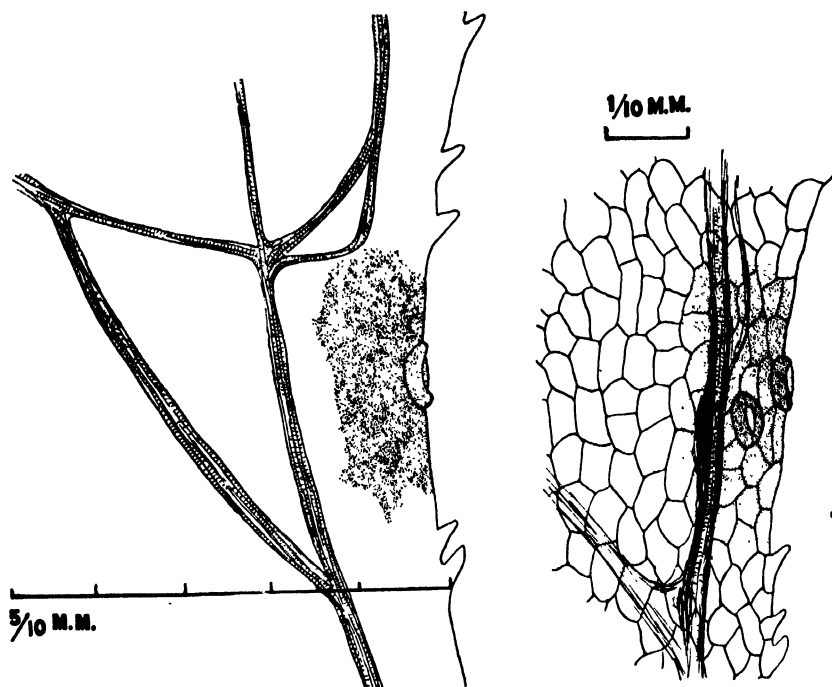


FIG. 8. Margins of mature leaflets, showing marginal vein and hydathodes. Dead tissue is indicated by the dotted areas.

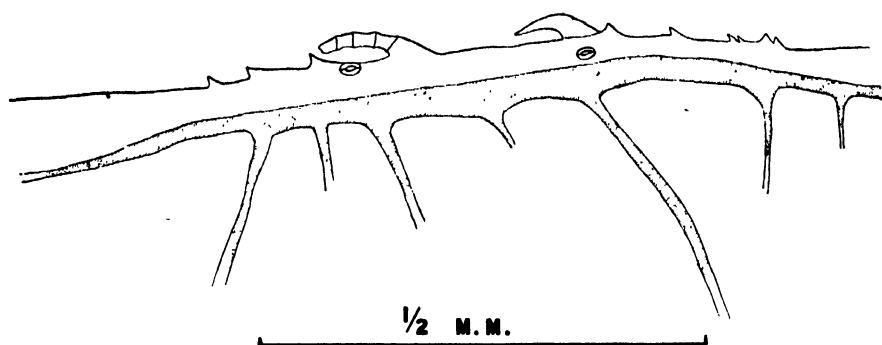


FIG. 9. Margin of leaf showing the extent of injury to the marginal and connecting veins. Injury is indicated by dotting.

Figure 1,B displays the superficial aspect of tissue death in the tip of the leaflet. The palisade parenchyma under the openings turn brown and, since the hydathodes are very closely distributed in this region, the whole tip soon becomes thus discolored. The tissues involved on the side of the leaflet may include the palisade parenchyma and also

the pulp cells at the margin or over the marginal vein (Figs. 8, 9) without the bast of the marginal vein becoming injured. If, however, the water pores lie very close to the vessels of the vein, the bast and the long cells which accompany the veins become browned and apparently die, especially with the older leaves. Figures 8 and 9 are illustrations from mature leaves. The tissues around the hydathodes on the young leaflets seem to have a better chance to escape injury especially if they lie at the bottom of a depression or well on the margin (Fig. 5, C) but the water pores which open to the outside without any chamber above them (Fig. 5, B) may show browning. The young leaflets are never as susceptible to tipburn as the older ones, hence it is doubtful whether cell death seriously damages the plant in view of the fact that the influence is mainly local. Figures 8 and 9 exhibit the extent of the injury to the marginal vein and, of course, to the portions of the leaf which depend upon it for water supply and for the removal of the products of assimilation. It would seem from these measurements that the localized damage due to the presence of the water pores does not extend into the margin of the leaflet for the distance of more than 1 mm. or possibly even  $\frac{1}{2}$  mm. This theory would be correct if the influence of the marginal vein did not extend beyond its immediate proximity, but this is not true, as will be shown later.

The death of the cells located under the water pores would seem to be due to excessive transpiration leading to so severe a plasmolysis that they are able to revive and resume normality. However, Morse's (17) observations touching the effect of borax applied in the fertilizer upon the rapidity of tipburn attack and the progressive and early death of the leaflets at least raise the question whether other factors may not be at work to bring about similar results. He says:

"Quite a different type of injury occurred upon all plants which were grown in pots (in the greenhouse) containing a fertilizer which carried borax.

"This type of injury was characterized by death and drying out of the tips and margins of the leaflets. The injury first appeared on the basal leaves and afterwards on the upper ones, and almost without exception was noted on the tips of the leaflets first and then on the margins. . . . In like manner the terminal and first two lateral leaflets were attacked first and were more severely affected. . . .

"The age of the leaf seemed to be a determining factor. A lower leaf might be badly affected while the leaves from a young shoot formed on its axil would appear entirely healthy at the same time. However, these leaves from the younger shoots nearer the base later showed the same marginal injury. . . .

"The appearance of the affected leaves seemed to indicate simply a progressive death and drying out of the tissues. While there was a fairly distinct line of demarkation between the diseased and healthy portion of the leaves, the latter near this line usually showed more or less fading out of the normal green color to a lighter green or even a yellowish tinge. . . .

"It seemed reasonable to suppose that compounds of boron were being taken up by the roots, were being carried along with dilute solutions of food materials and deposited in leaves. . . .

"The sample in the injured leaves gave a positive test (chemically) for boron while that from the healthy leaves gave a negative test."

Neller and Morse (18) have confirmed these borax results in an elaborate series of greenhouse experiments conducted at the Vermont Agricultural Experiment Station on a variety of plants.

Similar observations were made by the writer on the severe attacks following the use of a chemical compound in the potash which caused the plants to rot off before reaching the surface of the ground. This chemical was not borax nor any of the borax compounds according to the statement of the Chemist of the Vermont Agricultural Experiment Station and of the Chemists of the Bureau of Soils, of the U. S. Department of Agriculture, at the time, but was not identified.

Such occurrences of course call for explanation. Spanjer (22, p. 75) having kept a plant of *Phaseolus multiflorus* for 24 hours in a 0.06 to 0.05 per cent solution of eosin, under pressure, noted that the guard cells of the water pores become colored and then collapsed and closed. If evaporation were rapid, the water would be quickly removed from the first faintly tinted drops and the percentage of dye in solutions would increase and even if the first drops which came through were not toxic, they might rapidly become so. A similar process probably goes on if solutions which contain traces of toxic substances such as the boron compounds are absorbed by the roots and are later extruded by the water pores. Even if the percentage of toxic substance were not high enough to kill the guard cells at once, the limits of toleration would soon be passed and the guard cells would be poisoned. The percentage of borax absorbed in the soil water from fertilizers maintaining traces of it must be exceedingly minute but in the margins of the leaf it is comparatively easy to obtain chemical tests for it.

It is possible, too, that even if the guard cells were not killed by toxic substances, they may be poisoned to such an extent that they are unable to close at times when water losses from evaporation become excessive. In fact, tipburn may be due to such a paralysis of these organs. Neigh-

boring fields apparently similar in all respects are very differently affected during a tipburn epidemic, and this suggestion would seem to offer an explanation of such an occurrence. In the writer's experience, however, the heaviest foliage losses occur on fields that are exposed to the direct rays of the sun, that is that slope toward the south, especially if they are so located that the unobstructed wind can sweep across them. It is his judgment that the toxic effect is likely to be secondary in most instances, although undoubtedly at times it is a primary factor.

Some of the older experiments by which liquids were forced through the water pores under pressure of a mercury column were repeated on branches of the potato plant. Under a 30 to 35 cm. pressure, it was possible to force water out of the pores in about two hours. Fair-sized droplets were formed, often five or six to a leaflet, and two or three droplets on the small intermediate leaflets. These droplets were rather evenly distributed along the margins. The distribution of the water pores is such as to have led one to expect that they would have tended to form toward the tip; however, the point of least resistance seems to be the determining factor, and this may be as well at the side or back of the leaflet as toward the tip. A very weak eosin solution was forced through the pores in two hours, but it was about 24 hours before the yellow color appeared. The leaf tissues were flooded with the solution, and the leaves drooped and wilted in a few hours afterward. No special region was noted as especially susceptible to flooding. A 2.5 per cent solution of copper sulphate was forced through in another plant, and the copper reaction was obtained in the drops within 24 hours. The branch thus treated suffered even more severely than did that treated with the eosin solution, and the leaves were black and practically dead at the end of 36 hours. Haberlandt's experiment was repeated, in which the margins were painted with a 0.1 per cent solution of mercuric chlorid in 5.0 per cent alcohol before being subjected to pressure. The margins promptly darkened, and the water collected along the margin in light greenish yellow drops. It is a moot point whether these drops were secreted through the pores or through ruptured leaf tissues. Spanjer (22, p. 75) was able so to poison the guard cells of the water pores of *Phaseolus multiflorus* with solutions of copper sulphate mercuric chlorid, or with iodine vapor that they collapsed and closed. The leaf tissues were filled with water in all their intercellular spaces, but only after 30 hours did water exude from the pores. The droplets which then appeared undoubtedly had broken through the epidermal tissues somewhere in the neighborhood of the water pores.

The marginal anastomoses which really form the marginal vein



probably play more of a rôle than its size would indicate although it is one of the largest of the anastomoses in the vascular system. This rôle is the equalization of the water supply to all parts of the leaf, since this vein comes into contact with all the lateral veins. Haberlandt (9) found that severing one or two of the principal veins in the leaves of the sycamore did not cause the death of those portions of the leaf supplied by these veins, since the tissues seemed able to derive their water supply from other sources by means of the abundant anastomoses. In fact, such leaves remained to all appearances uninjured, living as late in the autumn as did other leaves. However, he is careful to point out that while new tracheidal connections may be formed, such changes involve considerable delay, during which the portions of the plant which have been cut off from their regular water supply are in danger of drying up. Such a condition might occur in the potato leaf whenever a very hot

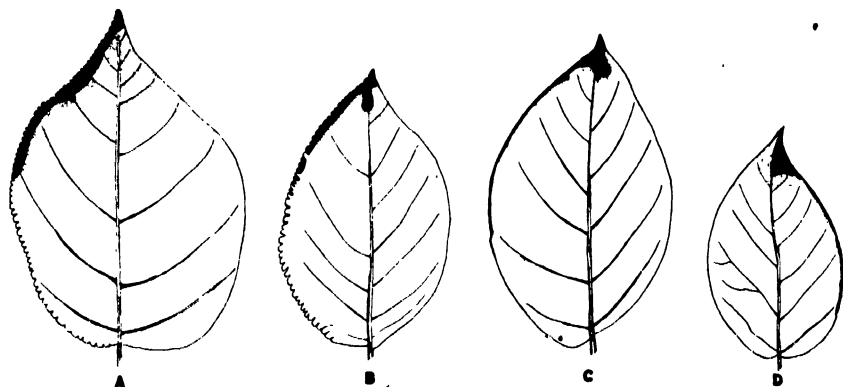


FIG. 10. A, B, Leaflets whose margins were clipped on August 25 and examined and drawn on August 29 after being exposed for four days to hot sunshine.

period intervened. These marginal tissues would be apt to die under the influence of the heat and the intense sunlight. Excessive transpiration in some one or more portions of the leaflet—as elsewhere described by the writer (12)—would necessitate an extraordinary water supply. The vessels which regularly serve these affected portions can not carry enough water; neither can a sufficient excess be brought by means of bypaths through the anastomoses of the marginal vein. As a consequence, the leaflet wilts, the cells are plasmolized and the chlorophyll and the chlorophyll bodies are destroyed. If the plasmolysis goes beyond a certain limit, the cells can not recover and local death ensues. An explanation of this sort would seem to account for the advance of tipburn beyond the margin of the leaf; and since in some cases physiological tipburn involves half or more of the entire area of the leaflet, some

adequate reason must be advanced since the damage immediately under the water pores is slight and localized.

On August 25, 1920, at 2 p. m. the marginal veins of a few Green Mountain potato leaflets were cut in about 20 places along one side of the margin only. The cuts were made with a safety razor blade and were not over 1 mm. long. The weather was intensely warm in the sun

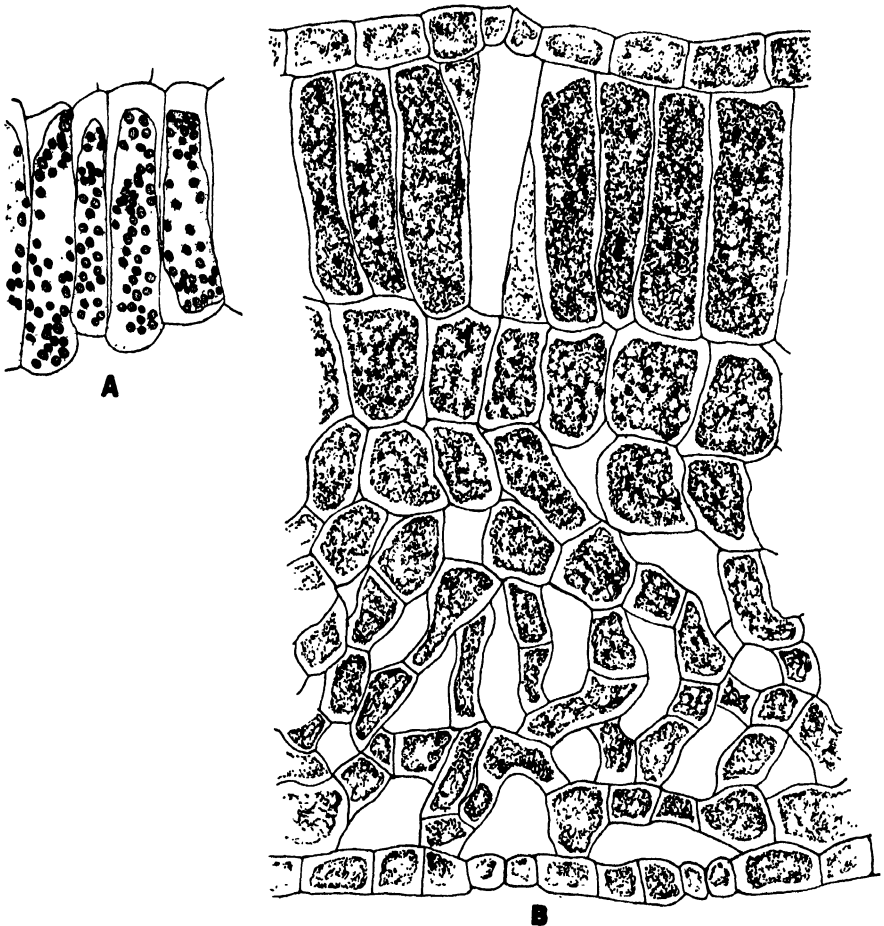


FIG. 11. Section from a living leaflet, showing the cells of the area wilted after a very hot day (July 22). A is from a leaflet not as severely wilted as B. B will not recover; A will be yellowed.

and the plants showed signs of wilting. These leaflets on August 29 showed typical brown tipburn along the cut margins as well as the distinctly yellow margin characteristic of typical tipburn (Fig. 10, A, B). Severing the marginal vein in a number of places at a critical time will lead to tipburn. A similar experiment was tried with other leaflets,

1 mm. of the margin being shaved off, with similar results (Fig. 10, C, D):

The sequence of events in the death of the margin of the leaflet is probably much more complicated than the foregoing experiment would indicate. The dead cells surrounding the vein serve as a barrier to the outward passage of the water from the vessels into the leaf tissues, and the latter gradually dies during hot weather. The margin of the leaf finally collapses when it becomes brown. The marginal vessels are choked, no longer act, and then we have the loss of the important regulatory function described above.

The condition of the plant during a severe tipburn attack has been elsewhere described, (12) but it may be well to recall the main features of this disease. Those portions of the leaf upon which the sunlight falls at right angles to the leaf surface become wilted and yellowed. The other portions escape but, following change in position of the sun, other margins and tips present themselves successively to the right angled sun's rays and in turn may become wilted, while these portions subjected to this condition earlier in the day may recover their turgidity. A section of the leaf taken at this time while it is wilted, (Fig. 11) shows all the cells plasmolyzed and to about the same extent. The question naturally arises: why does plasmolysis occur only in the portion of the leaflet upon which the sunlight falls perpendicularly? The explanation appears to be in the peculiar form of certain cells. The palisade parenchyma cells are long and narrow and are lined with chlorophyll bodies along the sides. These absorb the sunlight and protect the underlying pulp parenchyma cells. As long as the leaf can protect its pulp parenchyma, it does not wilt badly, but any prolonged exposure of its under tissues to clear, hot sunlight promotes transpiration to an extent that the plant is unable to transmit enough water to this localized region to maintain cell turgidity. The portions that are worst plasmolyzed die, while other parts less severely affected regain turgidity but may become yellowed as a result of the destruction of the chlorophyll. Sun plants are always lighter in color than are shade plants of the same species.

The potato plant seems peculiar in respect to the amount and extent of the injury to which it is subjected by tipburn of the physiological type. The serious damage is done when the sun's rays fall on the tips and margins of the leaflets at such an angle that they are parallel with the side walls of the long palisade parenchyma cells. Many field plants, such as the pea and bean, assume a "sun position" in which the margin of the leaf is turned toward the sun, thus protecting themselves against damage. The diurnal movements of

*Helianthus* are probably for the same purpose. Wiesner (26) has expressed this relation of strong sunlight to plants, especially the tropical species, in the following principle: The adaptation of the plant to the direct sunlight expresses itself in such a way that the green vegetative organs, i. e. the leaves, avoid all direct sunlight of great intensity and take up only direct light of lesser intensity. He cites *Robinia pseudoacacia* as an example of a plant which receives direct sunlight at right angles; that is to say, direct sunlight causes the leaves to turn their edges to its rays. Many tropical trees hold their leaves straight up in the air in order to avoid the direct sun's rays. The potato plant, however, seems to exhibit no such attitude. Unlike those mentioned it is unable to avoid these destructive perpendicular rays. Hence any portion of the leaflet upon which they fall must needs suffer until the movement of the sun alters the angle when, in turn, other portions of the leaf or plant may be thus affected. In the meantime, however, it often happens that the chlorophyll has been destroyed and the cells plasmolyzed to such a degree that they can not recover.

The ease with the older leaves become plasmolyzed is increased by their lowered osmotic pressure as compared to that of the stems. During these periods of intense sunlight, the plant is forming starch in abundance and the stem juices are loaded with sugar which is being transferred to the tuber for storage as starch. The osmotic pressure data obtained during 1918 (13) have been supplemented during the past two years as a result of further observations still unpublished, wherein it was found that the pressure in the stems often was approximately as great as in the leaves.

Certain further observations on the chlorophyll content of the cells may have some bearing on the ease with which the older leaves are affected by too intense sunlight. The young leaves in the center of the plant are much darker green, in early July before any signs of tipburn appeared, than are the older leaves that are beginning to droop. If like weights of each are taken, boiled, and their chlorophyll extracted in alcohol it will be found that the tip leaves solution, diluted twice or thrice, is as intensely green as that from the older leaves. The augmented chlorophyll content of these tip leaflets served as an additional protection against cell destruction.

In this connection, too, it may be of interest to note that much of the increase in size of the leaflets is due to swelling of the cells, as the following data show.

	Area (sq. mm.)	Length (mm.)	Breadth (mm.)
Epidermal cells from underside of tip of young leaflet	0.0045		
Stomata from underside of tip of young leaflet		0.0033	0.0026
Epidermal cells from underside of tip of old leaflet	.00115		
Stomata from underside of tip of old leaflet		.0039	.0027
Epidermal cells from upper side of tip of young leaflet	.0007		
Stomata from upper side of tip of young leaflet		.0036	.0026
Epidermal cells from upper side of tip of old leaflet	.00205		
Stomata from upper side of tip of old leaflet		.0045	.0031

The amount of chlorophyll in the young leaves being two or three times that of the old leaves, bulk for bulk, it would seem that the chlorophyll per cell content does not increase very much during the period of the leaf expansion.

A study of the leaflets which have been subjected to severe plasmolysis but which have apparently recovered shows that many do not attain normality but, on the contrary, are very susceptible to further excessive transpiration losses. A yellow or yellowish green region usually surrounds the brown and dead tissue and the chlorophyll is much less and the chlorophyll bodies are smaller.

If the leaves are cleared with glycerin, it is possible to observe the affected tissues. The plant hairs seem to succumb most readily, that is, they are more easily and more seriously plasmolized than any other tissue unless it be the bast of the veins. The veins which extend into the green tissue from the brown and dead area show a light brown bast along the vessels. Occasionally, on some leaflets, the stomata shows brown guard cells and the underlying tissue is killed for the distance of a few cells from the air chamber. Occasionally two stomata in close proximity to each other are connected underneath by dead cells along the air spaces which connect them (Fig. 12). However, these conditions are not often observed and the advance of the tipburn into the leaflet is to be expected along the veins. In a few instances, too, some of the irregular pulp parenchyma cells touching the bast are browned.

Sections of the leaflets from mature plants taken from the field during August were placed in 4.5 and 5.0 per cent solutions of potassium nitrate, the air was removed so far as possible by exhaustion with a pump, and the leaflets were examined after about an hour's immersion. The

cells of the hairs were found to be plasmolyzed severely, as were the sieve-tubes and their companion cells. The irregular pulp parenchyma was slightly plasmolyzed, while the long palisade cells seemed to be almost unaffected. These facts tend to corroborate the theory that the death of the leaflet is due to extreme and repeated plasmolysis and wilting.

Particular care has been taken thus far in the discussion to avoid all mention of the injuries due to insects, especially the leafhopper. The reason for this silence has been that the only phase of the tipburn problem under consideration was physiological in its nature and not due to insects in any way. The almost entire absence of leafhoppers from Vermont fields eliminates them as a factor and makes the solution of the problem that much simpler. During the latter part of July and the early part

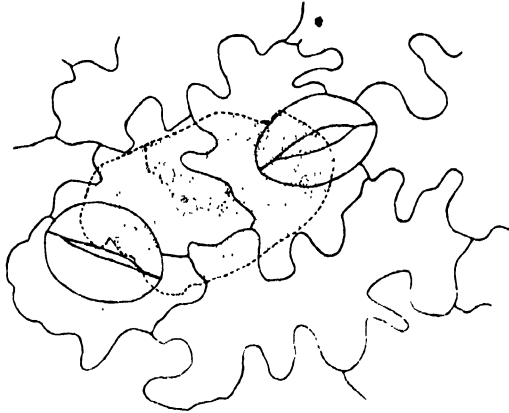


FIG. 12. Cells under adjacent stomata (killed portions indicated by dotting) but in an area that has apparently recovered from mild tipburn.

of August, one or two leafhoppers could be found on a plant if careful search was made, but in about a week (July 20 to 27,) the potato plants lost at least a third of their foliage from physiological tipburn. This fact in itself is sufficient answer to those who doubt the field occurrence of this type of tipburn. Similar weather was experienced in 1917, for example, and the same type of tipburn occurred.

During August, the number of leafhoppers continued to increase until 10 to 15 could be scared from a plant, but the tipburn remained almost stationary in extent. The weather was cool and the sunshine neither hot nor brilliant. A few days of warm weather in late August (20 and 27) brought about considerable addition to the tipburn area. The author believes that the presence, field importance, and physiological origin of tipburn has been proved.

On the other hand, the relation of the leaf hoppers to what has been called tipburn in regions where these insects are so abundant that they fly up in clouds when disturbed is not so clear. The experiments which the entomologists have made are all aside from this point, since they avoid or are unable to produce field conditions in the screened plants with which they have worked. Their usual procedure has been to put a single plant under a screen without leafhoppers and another plant beside it under a similar screen with them. Any field ecologist in botany would tell them at once that the intensity of the sunlight is greatly diminished under any sort of screen and that the wind velocity has been reduced to almost nothing. Both these factors reduce transpiration, and loss of water is the essential factor in tipburn of the type now under discussion. The vital question is: Would the plant with no leafhoppers tipburn physiologically if left under real field conditions? It would be hard to devise a procedure that would separate the two factors, but until the entomologists are able to do this, it must be assumed that physiological tipburn is the original type and that in most instances the leafhopper is only an aggravating and serious factor. Under similar conditions, the aphids might contribute in a similar manner, as is indicated in "The Report on the Occurrence of Insects and Fungus Pests on Plants in England and Wales" for the year 1919 (6) which says: Potatoes were noteworthy in 1919 as having suffered from a very general attack by aphids which was followed by various obscure troubles apparently correlated with the aphid attack. It has not yet been shown whether the progressive death of the tissues surrounding the original puncture injury is due to a toxin introduced by the aphid, but it seems likely, since the potato shows a very marked reaction to the toxin introduced by species of Capsids. Or, to go back still further, Smee (21) attributed all potato diseases to aphids, especially the blight which was at that time the subject of discussion and investigation, since the causal organism had not then been discovered. Smee describes similar diseases on turnips, black nightshade, belladonna, stramonium, horseradish, tomato, nettle, mallow, etc., all induced by these same active insects.

The type of injury attributed to the leafhopper is a puncture which partially severs some of the more important veins. The leaf collapses and, sooner or later, the lamina of the leaf supplied by the vessels dies and becomes brown, this browning area having the shape of a fan with the broad portion on the margin. According to various entomologists, it makes little difference how dark it is or at what time of year the trial is made. The injury is so severe that the leaflets gradually die until

the plant is only a bare stalk. It is a question whether the severance of a single vein, even of good size, would cause the death of the tissues which it ordinarily supplies with water, but if the connection is broken during very hot weather, when all the water tubes, working at full capacity can barely supply all parts of the leaf lamina, the tissues might well become plasmolyzed and collapse. In the former cases, the anastomoses would serve as substitutes, but in extremely hot weather these bypaths would not suffice.

Haberlandt's experiment in which he severed some of the large lateral veins was repeated on August 25, using several potato leaflets. The leaves were again examined on August 29 but nothing had happened, the tips, margins and portions of the lamina which are supplied with water by these veins being as alive and healthy as on August 25. The sunshine had been brilliant and the temperature high, but the anastomosing veins had kept the tissues supplied with enough water to prevent severe plasmolysis and death during a fairly critical period when tip burn was advancing.

The portion of the leaflet supplied by the vein that had been severed by the leafhopper puncture might appear to die from the insect attack and still the death might really be brought about by the physiological form of the disease; for during the weather which favors insect attack, physiological tipburn would undoubtedly make its appearance and break down the anastomoses, since field conditions so far as heat and sunshine are concerned are much the same throughout the United States. It is almost impossible to separate the two factors if they occur together, but it must be conceded that the injury which the leafhoppers do to the plants must be serious when it will produce a burning of the leaves in a cage in partial shade.

The yellowing of the chlorophyll in the region of a tipburn area is to be regarded as a physiological symptom, as the writer has already pointed out. Intense illumination will break up chlorophyll and increase the percentage of carotin and, so far as is known, the chlorophyll is not able to regenerate itself (23, p. 92). This destruction of chlorophyll to form yellow pigment seems to be going on all at times in green plants in the sunshine, but those plants which have devised some means of preventing a too rapid destruction have survived. Sun leaves always exhibit a yellow-green hue as compared to the intense blue-green of shade leaves. The entire plant might be yellowed as a result of the sucking of the plant juices by insects, but it is hardly likely that the yellowing would be limited to a line bordering the dead area.

An experiment was described in a previous paper by the writer (12)



in which tipburn was produced in a very short time by the exposure of greenhouse plants to the action of an intensified sunlight produced by a series of three mirrors. This was done in April, obviously a time of year when tipburn does not occur on such plants. Similar experiments were conducted during the following spring with a somewhat different arrangement of mirrors. These mirrors, each 2 feet long, were so placed one above the other in a frame that they could be tilted. It was thus possible to subject a leaflet for at least a half hour to an almost threefold intensity of sunlight. The burns that were produced simulated in many ways the browning brought about by sunlight and heat in late July, but with some slight differences which had not been noticed in the previous paper. Any portion of the leaflet could be subjected to the intensified light and plasmolyzed to such a point that recovery was impossible. The leaflets showed brown areas over at least a part of the treated regions. These brown areas, however, were rather sharper in these margins than in natural tipburn produced in the middle of the summer, the typical yellow margin being absent.

The tipburn that appears early in the season partakes of the nature of this artificially produced scorching. After a season of lush growth, with tender foliage produced during fairly cool weather with an abundance of moisture, a few hours of hot, brilliant sunshine will scorch almost any portion of the plant upon which it may happen to fall directly for any length of time. Such a period was noted during the present year about July 3. The damage done was considerable but was not to be compared with that which came on about two weeks later.

The writer, therefore, is inclined to revive the distinction heretofore made by some of the older writers and to recognize two forms of physiological injury to the potato plant due to heat, sunlight, and excessive transpiration. The one type is caused suddenly and disappeared abruptly, presenting rather clear lines of demarcation and attacking even the younger, succulent leaves. The other type is the result of several days of warm, dry sunshine, usually shows tissues surrounding the dead portion that are diseased and appear yellow, and is more common on the middle-sized and older leaves. Intermediate forms undoubtedly appear, and there really is very little essential difference in them, since extreme plasmolysis is at the root of the injury in either instance. In tipburn, however, the plasmolysis is repeated and its influence extends somewhat beyond the area where it causes death.

The stomata on the potato leaf undoubtedly play an important rôle in the transpiration of water; hence some attention was given to these organs as well as to the water pores. De Vries (25, p. 605-506) says

in translation: "The epidermis of the potato leaf bears stomata both above and below, though on the lower side considerably more than on the upper side. The number per square millimeter varies according to the environment, so that at times the upper surface is found to be entirely without stomata. Czech (4, p. 842) found 0-2 per square millimeter, while I have found on one specimen out of the garden an average of 10 and on some other plants grown from seed, 20 stomata on the same area. On the under side Morren (16) and Czech (4) agree on 263 stomata per sq. mm. It would not be impossible that here, also the environment has something to do with the number of stomata, as Czech has observed the fact that the varieties which grow in damp situations have more stomata than related species which occur in dry places. I have found the end length of the stomata to be 0.045 mm."

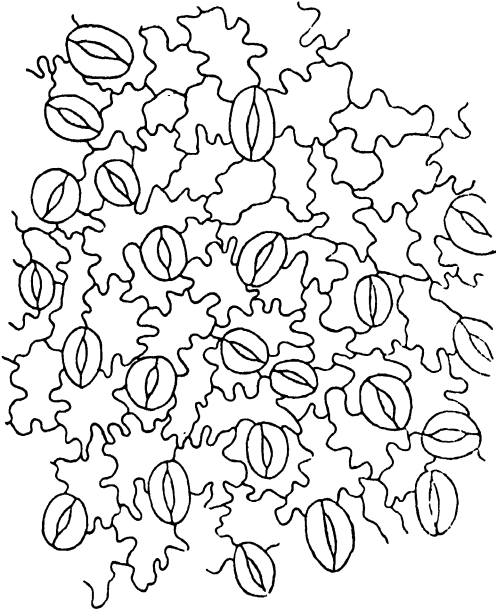


FIG. 13. Upper side of young leaflet: 230 stomata in 1 sq. mm.. x200.

The counts given below are from Green Mountain potatoes growing in a rather dry soil during a very dry summer (1921), the counts, with one exception (Sept. 2) being made during August. Tip leaflets were used (Figs. 13, 14, 15).

	Stomata per square millimeter	Stomata on an average leaflet
Young tip leaflet:		
Under side, middle region.....	300.....	82,800
Upper side, middle region.....	230.....	52,900

Medium size tip leaflet

Under side.....400.....348,000

Medium size tip leaflet

Upper side:

Middle region.....140.....	} 95,700
Basal region.....80.....	

Large size tip leaflet:

Under side:

Basal region.....115.....	} 242,000
Apical region.....105.....	

Upper side:

Basal region.....60.....	} 99,000
Apical region.....30.....	

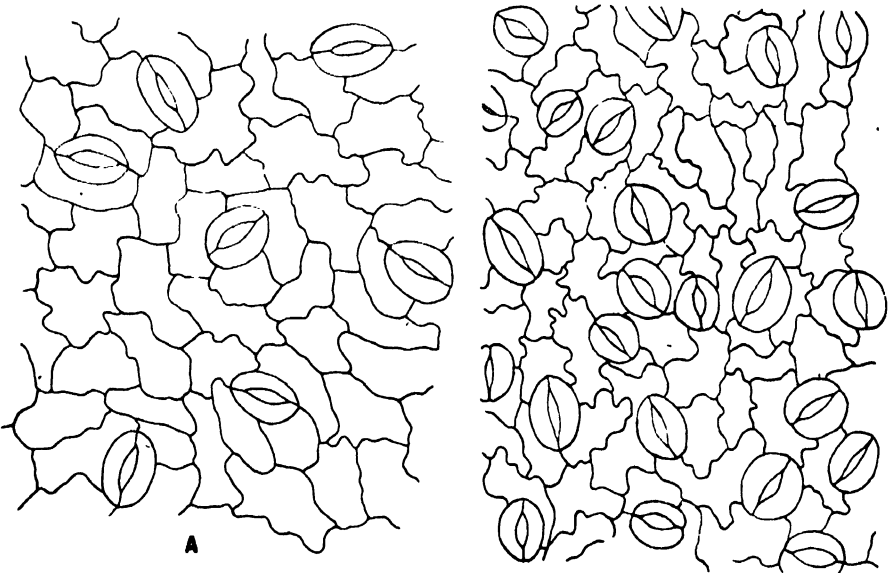


FIG. 14. A. Upper side of medium-sized tip leaflet with 140 stomata in 1 sq. mm. x200. B. under side of medium-sized tip leaflet with 400 stomata in 1 sq. mm. x200.

Clearly the numbers vary on different portions of the leaflet, a very large proportion being located on the upper side of the leaflets. On the young leaflets 39 per cent are on the upper surface and 61 per cent on the lower on the medium sized leaflets, 21 per cent on the upper and 79 per cent on the lower; while on the large tip leaflets the percentages are respectively, 20 and 71. The young leaflets are held upright at a



FIG. 15. A. Upper side of old tip leaflet, near base of lamina with 60 stomata in 1 sq. mm. x200. B. under side of old tip leaflet, near the base of lamina with 115 stomata in 1 sq. mm. x200.

very acute angle to the sunlight, whereas the medium and large leaflets droop. The formation of stomata seems to cease on the upper side some time before it does on the lower. The young leaf in its growth increases in breadth much faster than it does in length, so that the leaf lamina expands and the tip of the leaf instead of being shaped like a spear point assumes the form of a broad oval. In this connection it is interesting to note that Miss Rea (20) found about three times as many stomata on the under surfaces of leaves from normal shoots of *Campanula rotundifolia* L. as on the upper, while the shade shoot leaves have more than three times the number on the under side as compared to the upper and the sun shoot leaves show numbers approximately equal on both sides.

In conclusion, the relation between the practice of spraying and the water pores, the marginal vein, and the tipburn and margin-burn to which they lead might be pointed out, although this has already been done by Clinton (3) in the paper previously cited. The gelatinous films which constitute bordeaux mixture flow down to the edge and tip of the leaf and effectually stop up the air pores. It is very doubtful whether they can open at all when they are thus sealed up by the thick layer which is usually found around the margins of the leaflets. It constitutes a hard and fairly permanent deposit, comparable in a way to limestone, rather than to the sediment which Clinton (3) has in mind. Lime-sulphur forms no such thick and tough layer. Its very fine granules or crystals are of no avail in stopping up the water pores and hence would not act as a deterrent in the early stages of physiological tipburn. In view of the fact that the pores are always located on the upper side of or just over the margin toward the upper side of the leaf, such a stoppage is possible, whereas if they were located on the under side the spray could not reach them and they would remain open. The Bordeaux mixture also covers a large part of the stomata which lie on the upper side of the leaf—about one-fifth of them are thus located—and the loss of water must be considerable when they are in active operation.

The shading effect of Bordeaux mixture has been discussed by a number of writers. It should be remembered that this shading is most marked along the veins where the mixture tends naturally to flow and to dry. The importance of this fact is readily seen when it is recalled that the bast cells of the veins and the neighboring pulp parenchyma succumb when the wilting in localized areas becomes so marked that the cells are plasmolyzed to a point that they can not recover. The denser shade probably helps at these points. The shading also helps in providing the plant with a screen through which the sun's rays must filter before affecting the chlorophyll. Hence the destruction of the

chlorophyll to form carotin would be retarded and the light which entered the palisade cells, instead of falling on straight lines, would enter them in a diffused condition and the damage to the underlying tissues would undoubtedly be less.

#### SUMMARY

1. The tipburn discussed in this paper is entirely distinct from that form due to insect injury, such as the hopper burn due to the attacks of leafhoppers. Such insects are very rare in Vermont fields during the time when tipburn is at its height. This type is associated with hot, dry weather and clear, brilliant sunshine.

2. Potato leaves are provided with hydathodes around their margins but especially toward the tip. These hydathodes resemble the stomata but are placed so as to open directly on the vessels of a large vein which runs close to the margin of all the leaflets. A group of these hydathodes is located just at the tip of each leaflet where many of the vessels of the marginal vein end.

3. Tipburn begins beneath these hydathodes especially at the tip of the leaflets. The death of the tissues under the terminal water pore group leads to the browning and shriveling of the extreme tip of each leaflet. The tissues die under the water pores along the side of the leaflet and apparently break the continuity of the water supply system of the marginal vein.

4. The further advance of the tipburn into the leaf is due to direct sunlight acting on the cells of the leaf leading to such an extreme plasmolysis that the cells are not able to recover. Associated with this plasmolysis is the destructive action of the sunlight upon the chlorophyll bodies and the chlorophyll itself. This expresses itself in the yellowing of the green portion of the leaf in the region dead from tipburn.

5. In certain cases, such as poisoning from borax compounds in fertilizers, the death of the cells under the water pores is undoubtedly the result of an accumulation of the toxic salts in or around them in the intercellular spaces. It is possible, also, that the toxic effect is more marked on the guard cells of the water pores and that they are unable to function normally, that is to close or open.

6. A larger number of the stomata are located on the upper surface of the potato leaf than is generally supposed, varying from about one-third on the young leaves to less than one-fourth on the older ones. The tipburn seems to have little effect on the stomata or the tissues under them. Although occasionally they function in the browning and death characterizing this malady, as a rule stomata can practically be disregarded in the advance of the tipburn.

7. Another type of tipburn, more in the nature of a scorch, seems to occur especially on the young succulent foliage. This type may be seen at times in June or early July whereas the type described above is more common after July 20 in this region and is at its height about August 10. This form of tipburn may be produced by excessive sunlight in the laboratory at any time by the use of mirrors.

8. The injured tissue in the portion of a leaflet which seems to have recovered from tipburn is the long bast and companion cells and some of the adjacent cells of the pulp parenchyma.

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# RHIZOCTONIA SOLANI AS A POTATO-TUBER ROT FUNGUS

M. SHAPOVALOV

WITH PLATE XXIII

Though a well recognized parasite of the potato plant, *Rhizoctonia solani* Kühn is not ordinarily thought of as the cause of any type of tuber rot. Indeed, it is difficult to induce this organism to decompose any appreciable quantity of normal healthy potato-tuber tissues when inoculated with pure cultures. Various forms of *Rhizoctonia* injury have been reported as occurring in nature, but none could properly be called rot or decay. Nevertheless, there is good evidence at hand to justify the inclusion of this widely distributed soil organism in the list of potato-tuber rot parasites. However, it is only under special environmental conditions as well as in special abnormally constituted parts of the host that this decay takes place. So far, it has been observed by the writer only in irrigated sections of the West and only on elongated pointed-end tubers of the Netted Gem and Burbank varieties. The abnormal stem ends of these tubers (occasionally also knobs and eye ends) appear to be susceptible to the *Rhizoctonia* infection. When the latter takes place, a very peculiar jelly-type decay develops.

Carpenter<sup>1</sup> and Pratt<sup>2</sup> attributed the so-called jelly-end rot primarily to *Fusarium radicola* and *F. oxysporum*, indicating, however, that other factors may in part be responsible. It is true that various forms of wet stem-end rot prevail in the irrigated soils of the West which are quite thoroughly infested with several parasitic species of *Fusarium*. Naturally, these fungi may be most frequently found associated with stem-end rotting tubers, chiefly as primary, but occasionally as secondary invaders. Yet, cases are not infrequent, especially at harvest time, when elongated Burbanks or Netted Gems show stem-end decay of the jelly type while being entirely free from the *Fusarium* infection. Isolations from such tubers have shown that *R. solani* is the only parasitic organism present.

During the surveys of western potato diseases in the summers of 1920 and 1921, the writer had an opportunity to collect various stages of

<sup>1</sup> Carpenter, C. W. Some potato tuber-rots caused by species of *Fusarium*. Jour. Agric. Res., 5: 183-209, pls. A, B, XIV-XIX. 1915.

<sup>2</sup> Pratt, O. A. A western field rot of the Irish potato tuber caused by *Fusarium radicola*. Jour. Agric. Res., 6: 297-309, pls. XXXIV-XXXVII. 1916.

the jelly-end rot on Burbanks in California and on Netted Gems in Idaho. Special care was given to select tubers which came from healthy vines and had no vascular discoloration. The consistency of such decay was distinctly jelly-like. The color in the first stages of the infection was nearly white, but as the decay developed it turned yellow and then light brown and finally brown. All the isolations from such jelly-rot specimens of the pointed stem ends yielded *R. solani*. Portions of the decaying tissues under the microscope showed the cells to be permeated with the mycelium of the fungus. This Rhizoctonia jelly rot does not usually advance very far into the tuber, but stops at a certain definite distance which varies for different tubers and apparently is determined by the extent of the abnormal elongation of the stem end. When this limit is reached, a sharp division between the healthy and the diseased tissue may be seen. The decayed end may be sloughed off when the tuber is just taken from the ground or it may dry up, shrink and harden (Pl. XXIII figs. A and B).

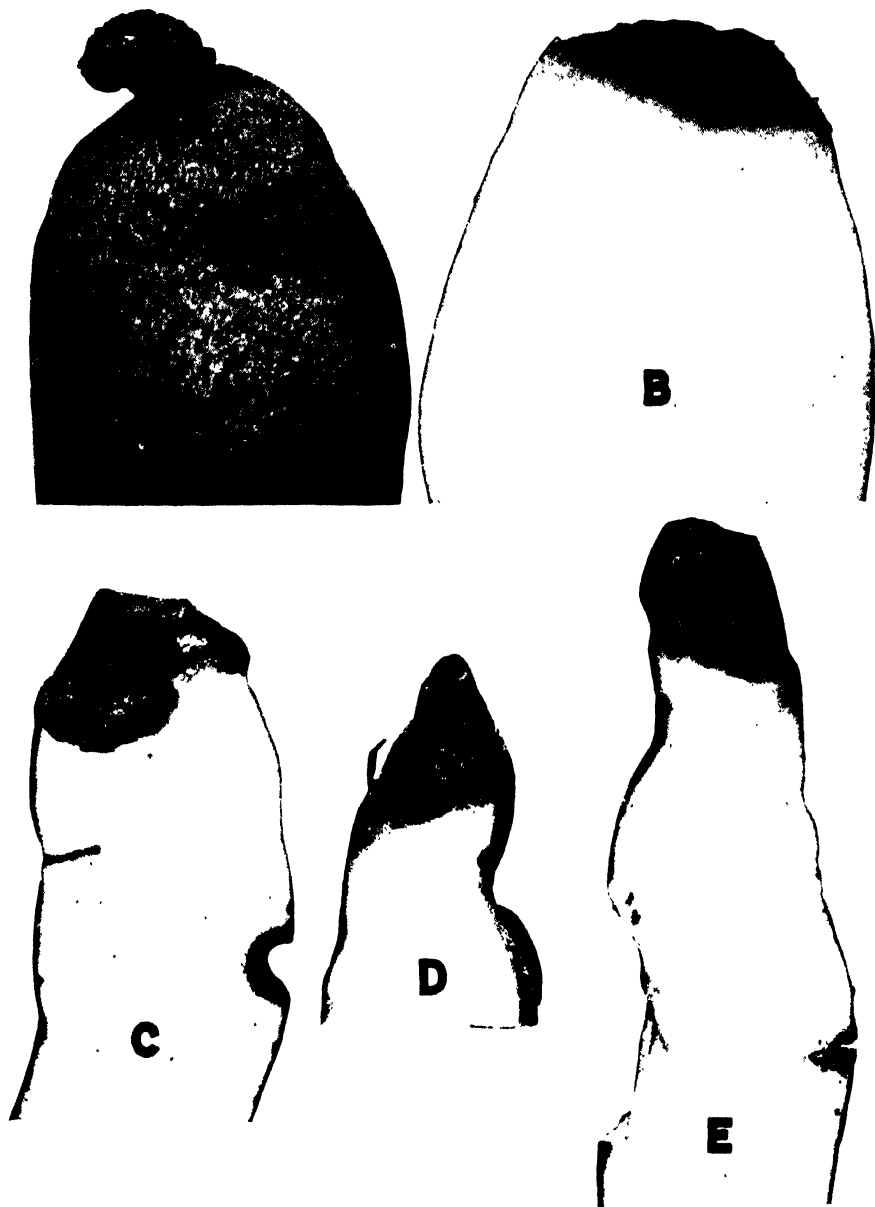
The flesh of the elongated stem ends, when cut open, differs from the remaining tissues of the tuber by a watery and somewhat translucent appearance. Under the microscope sections of these areas reveal a striking deficiency in starch contents of the cell. These stem ends may remain free from any infection, but they lose water in storage more rapidly than the rest of the tuber and subsequently shrivel up, though no decomposition of the tissue takes place. With regard to their internal appearance the elongated starch-free stem ends resemble most closely firm seed pieces found after the growth of the plant has been completed and all or most of the starch has been utilized. Such seed pieces, too, very often become affected with a jelly type of decay. Specimens of of this kind have been found by the writer in the vicinity of Washington, D. C., and in the greenhouses of the U. S. Department of Agriculture and species of *Fusarium*, *Vermicularia*, and *Rhizoctonia* have been isolated from them.

To ascertain further the possibility of a causal relationship between *R. solani* and the jelly type of decay of the elongated stem ends of the potato tuber, the following inoculation experiment was carried out. A thick layer of cotton was placed on the bottom of a glass moist chamber and covered with several layers of filter paper. Both the cotton and the filter paper were thoroughly saturated with boiled hot water and allowed to cool. Following this 5 abnormally elongated Netted Gem tubers, previously disinfected in 1 per cent formalin solution for 1 hour, were inoculated with *R. solani* isolated from the jelly rot material collected in the West in 1921. The inoculation was made both at the extreme

stem ends and on the sides, corresponding respectively to the abnormal and the normal tissues of the potato. Slits were made at these two points of each tuber and the *Rhizoctonia* mycelium from two-days old cultures on cooked potatoes was inserted in these slits. The inoculated tubers were then placed on the wet filter paper in the moist chamber described above and all were completely covered with wet sterilized sand. The chamber itself was left uncovered. During the first 10 days the tubers were kept in a refrigerator at night and in the laboratory room during the day at a temperature of 20°–25° C after which they were exposed for an additional 7 days in the laboratory during the day and night time. In this manner the material was subjected to all the variations in temperature such as may exist in nature. On the 17th day the tubers were examined and found to have each about 2.5 cm. of jelly-like decay at the stem ends and only a slight amount of soft but not jelly-like rot at the side inoculations (Pl. XXIII, figs. C, D, and E). *R. solani* was recovered from the jelly rot of the stem ends.

The results of this inoculation experiment as well as field observations and cultural work with field specimens show very clearly that *R. solani* is an important causative agency in bringing about the jelly type of decay of elongated stem ends of Burbanks and Netted Gems in the West, occasionally knobs and eye ends also being subject to the infection. Of course, this fact does not exclude the possibility of other organisms, particularly *Fusarium spp.*, causing a similar form of decay. Apparently, the jelly-like consistency is determined more by certain abnormalities of the host tissue, conspicuously manifest by a very low starch content or its entire absence than by the species of the parasite. So far as *R. solani* is concerned the extent of the abnormal structure of the tuber determines the progress of the decay, while *Fusarium* may advance further as a storage rot. The jelliness of the decayed portions disappears shortly after harvesting. The jelly-rot areas when dried and shriveled are externally indistinguishable from other forms of stem end rot, such as those caused by various species of *Fusarium* and in certain cases by the blackleg bacillus.<sup>1</sup> Consequently, it is rather misleading to apply the term "jelly-end rot" to any form of potato tuber decay either in storage or in transit and the broader term "stem-end rot" should be preferred in all those cases. Jelly rot or jelly-end rot designates an unstable form of field decay only. Soon after the tubers are put in sacks or in storage cellars this trouble passes into a general class of stem-end rot.

<sup>1</sup>Shapovalov, M., and Edson, H. A. Blackleg potato tuber rot under irrigation. Jour. Agric. Res. 22: 81–92. 1921.



JELLY-TYPE ROT CAUSED BY *Rhizactonia solani*:

A and B—Natural infection in the field, specimens photographed 5 months after harvesting: the decay was arrested and the diseased stem ends became shriveled and dry.

C, D, and E—Result of artificial inoculation with pure culture of *R. solani*, 17 days after the inoculation; C and E show also a very slow progress of the decay in the side inoculations.



# THE RELATION OF SOIL MOISTURE AND SOIL TEMPERATURE TO BUNT INFECTION IN WHEAT.

CHAS. W. HUNGERFORD

WITH FIVE FIGURES IN THE TEXT

## INTRODUCTION

Bunt, or stinking smut, is one of the most serious problems in the wheat growing sections of the Pacific Northwest. Nowhere else in the United States and probably nowhere in the world, with the possible exception of certain parts of Australia, are the annual losses from bunt as great as they are in the Palouse region of Idaho and adjacent Washington. In this region winter wheat is grown extensively, summer fallowing is practiced and there is a small amount of rainfall during the summer months. Due to these climatic conditions and cultural practices, the soil becomes infested with windblown spores of the bunt organism *Tilletia tritici* (Bjerk.) Wint. at threshing time. If the condition of the soil is favorable for germination of these spores immediately before or at the time of seeding, a very smutty crop may result although the seed may have been carefully treated.

In 1919, in coöperation with the Office of Cereal Investigations of the United States Department of Agriculture, a survey was made of cereal diseases in all of the grain growing counties of Idaho. In the counties where soil infestation by the bunt organism is known to take place, there was as high as 85 per cent infection in some fields. The average losses from this disease in these counties varied from 5 to 14 per cent of the total production. Some years the loss has been even greater.

It has been known for a number of years that soil contamination by wind-blown bunt spores is practically universal in the Palouse region. More recently it has been demonstrated that this condition exists in other regions as well. Barss (1) in Oregon and Mackie (12) in California have reported that soil infestation by bunt is common in certain parts of each of these states. The writer has found during the last three years that a like condition prevails in certain counties in southern Idaho where summer fallowing is practiced.

## VARIOUS METHODS RECOMMENDED FOR OVERCOMING SOIL INFECTION.

Various cultural practices have been recommended as aids in preventing infection from the soil. Heald and Woolman (6) in 1915 recommended, first, seeding before the threshing begins or if this is not possible

at least before the fall rains, second, reploting the summer fallow after the first fall rains, and third, late planting. Heald (5) suggests that under certain conditions it may be better to grow spring wheat instead of winter wheat, as spring wheat is not subject to infection from the soil. Heald and Zundel (7) recommend also crop rotation where summer fallow is not necessary for conservation of soil moisture and shallow seeding, as additional preventative measures. These recommendations have been made as a result of observations and experiments carried on at Pullman, Washington.

REVIEW OF RECENT INVESTIGATION UPON THE RELATION OF SOIL TEMPERATURE AND SOIL MOISTURE IN INFECTION BY VARIOUS FUNGI.

Within the last few years the question of the relation of the temperature of the soil to infection by various fungi has received considerable attention. Jones (9) calls attention to the importance of this line of investigation and reviews some of the more important literature. Gilman (3) has also reviewed the literature upon this question and states that *Fusarium conglutinans* Woll., would not produce the characteristic symptoms of cabbage yellows unless the temperature was above 17° to 22° C. Tisdale (14) working with flax wilt, Johnson and Hartman (11) with the root rot of tobacco, and Richards (13) with the *Rhizoctonia* disease of potatoes, have shown that there is a very marked influence exerted by the temperature of the soil upon infection by the specific organisms with which they were working. Jones (10) in 1895 called attention to the fact that soil temperature plays an important part in the amount of oat smut which may develop from a given seed lot.

Güssow (4) called attention to the influence of soil temperature upon infection by *Tilletia tritici* as indicated by certain field observations made by him in Canada. Woolman (16) in working with the same disease concludes:

"First, smutted grain planted untreated in smut-free soil, when the mean soil temperature is above 65° F. and sufficient moisture present to cause quick germination will produce a practically smut-free crop. Second, under the same conditions the per cent of smut will increase with the fall of temperature from 60° to 45° F., at which point the smut per cent is highest. Third, below 40° F. the smut per cent decreases with the fall of the temperature."

Jones (9) quotes Humphrey as stating, for the same disease, that "Soil temperatures of 0° to 5° C. and above 22° C. are decidedly unfavorable to infection." Walker and Jones (15), in a study of the relation of soil temperature to onion smut, found that under controlled

greenhouse conditions a large percentage of plants grown on smutted soil were infected at soil temperatures ranging from 10° to 25° C. A reduction in infection was noted at 27° C. and complete freedom from the disease resulted at 29° C. They also found that in out-of-door plantings a gradual reduction of infection occurred as the season advanced and the soil temperatures rose.

Although considerable work has been done in recent years in regard to the influence of soil temperature upon infection by various pathogenic organisms in the soil, very little attention has been given to the influence of the amount of soil moisture upon the development of such organisms. Heald and Woolman (6) in calling attention to the effect of the amount of moisture in the soil at planting time upon infection by the spores of *Tilletia tritici*, state, "Our experiments have given some evidence that planting when there is just sufficient moisture to induce germination is a good practice, and even that dry planting and waiting for a rain is better than planting in a very wet soil." Mackie (12) states, "Bunt spores require about 14 per cent of moisture in the soil or a moist atmosphere for germination" and that "soils may be moist enough to sprout the wheat while too dry to germinate bunt spores." Walker and Jones (15) have shown that soil moisture is not a limiting factor in the development of the onion smut fungus, *Urocystis cepulae* Frost. At a very high or very low moisture content they found a reduction in the amount of smut but this reduction was associated with a decrease in seed germination and rate of growth of the onion plants.

#### EXPERIMENTAL DATA

That the amount of moisture in the soil at planting time may have a very marked effect upon the amount of bunt in wheat has been shown in a striking way by observations which have been made in Idaho during the last 5 or 6 years. In the summer of 1919, Mr. A. E. Wade, at that time County Agricultural Agent in Lewis County, Idaho, called the writer's attention to observations which he had made. He observed several instances where there was a small amount of smut in part of a field, which had been seeded in dry soil before a rain, while in the balance of the field, seeded with the same seed after a rain, there was a much higher percentage of smut. A specific example cited by Mr. Wade follows. A part of a field which was seeded October 22 in dry soil produced only one per cent smut. Rain fell during the night of October 22, not enough, however, to keep the farmer from seeding again the next day. The same seed was used in completing the field



October 23 and this portion of the field produced 30 per cent smut. A number of similar cases have come to the writer's attention during the last 3 years.

The writer had endeavored to test under controlled conditions the influence of both soil moisture and soil temperature upon infection of wheat by *Tilletia tritici*. The following data give the results of these experiments.

#### METHODS

Jenkins Club wheat was used in all the experiments. This wheat is the most popular and the highest yielding wheat in northern Idaho, and is fairly susceptible to bunt. Duplicate soil samples were always taken at the depth the wheat was planted. Moisture determinations were made on a dry weight basis and the moisture equivalent is given for each different kind of soil used. The soil used in the greenhouse experiments was in each case composed of Palouse silt loam to which was added a small percentage of fine river sand. The moisture equivalent of this mixture was 20.7. The moisture equivalent of the soil in the field plots which were used was found to be 27.2. The moisture equivalent<sup>1</sup> was determined by the method given by Briggs and McLane (2).

In experiments where the soil was artificially smutted, spores were secured by grinding up smut balls and sifting the spores through a fine screen. The smut spores thus secured were thoroughly mixed with the soil before planting. In all greenhouse experiments and where soil containers were used in the field the soil was removed from the containers to a depth of 2½ inches and a uniform amount of smut was thoroughly mixed with each lot of soil before planting.

#### FIELD EXPERIMENTS

In the spring of 1919 an experiment was started at Nez Perce, Idaho, and duplicated at Moscow on the University Farm. Six soil containers were filled with soil containing different amounts of moisture varying from near saturation to less than enough to cause good germination of wheat. Large road tiles 3 feet long and having an inside diameter of 14 inches were used at Nez Perce, and galvanized iron cans of about the same size were used at Moscow. These were sunk in the ground so that the tops were just above the surface of the surrounding soil. The soil was heavily smutted and Jenkins Club wheat which had

<sup>1</sup> The writer is especially indebted to Professor Guy R. McDole, of the Department of Agronomy of the University of Idaho, for advice in connection with this work and for the actual determination of the moisture equivalent of the soils used.

been rolled in viable spores of *Tilletia tritici* was planted in each container. Soil moisture samples were taken from each container at planting time and again when the wheat had all emerged. The average of these two was used as the average moisture content of the container for the experiment. A canvas was spread over the tops of the containers in order to keep evaporation at the minimum. The graph in

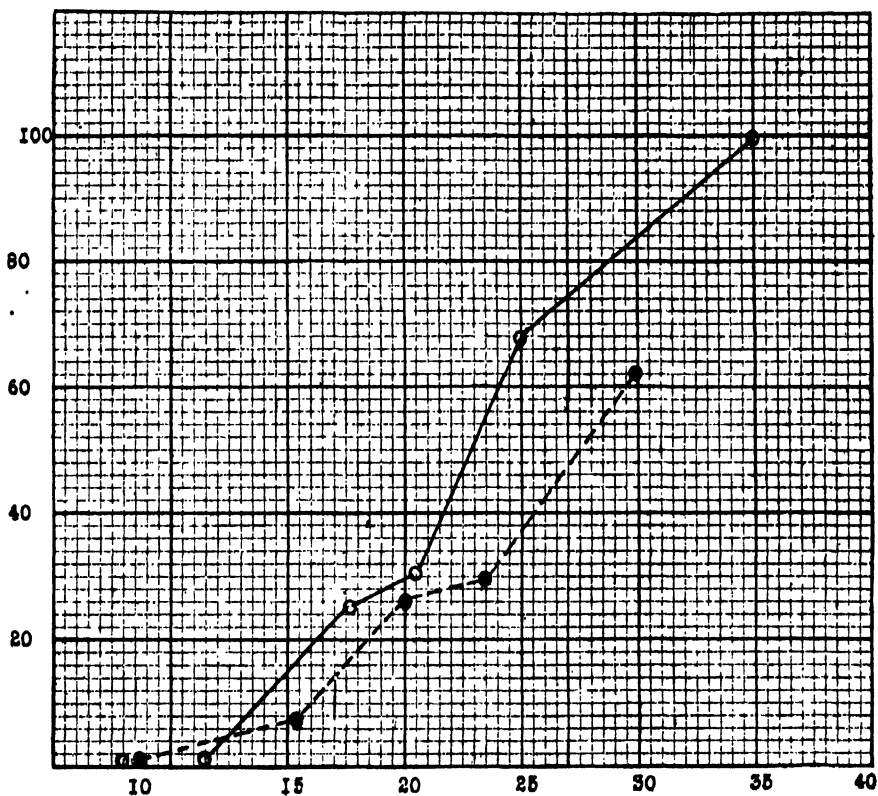


Fig. 1. Abcissa shows percentage of moisture in the soil at planting time. Ordinate shows the percentage of bunt infected plants. The unbroken and broken lines represent the results of the experiments at Moscow and Nez Perce respectively.

figure 1 gives the results of the experiment.<sup>1</sup> The soil temperature varied about 3 or 4 degrees F. in the several containers. The dryer soil had a higher temperature in each case. At Moscow the soil temperature varied from 48° to 60° F. during the time of germination of the wheat. At Nez Perce the variation was from 42° to 60° F.

Twelve circular plates 1½ inches in diameter were made of Plaster of Paris and viable spores of *Tilletia tritici* were placed on the flat sur-

<sup>1</sup>A brief abstract of this experiment has already been published. (8).

face of these plates. These were tied together in pairs and a pair buried in the soil in each soil container used in the above experiment at Moscow. When the wheat had emerged these plates were taken out and the spores were examined for germination. No germinating spores were found on the plates from cans numbers 1 and 2. On the plates from can number 3 a very few germinating spores were found; on those from cans numbers 4 and 5, slightly better germination was found; and about 25 per cent of all spores were germinated on the plates from can number 6.

An extensive series of tests was outlined in the fall of 1919 and again in the fall of 1920 in which series of rod-row plantings and one-fortieth acre plantings were to be made at intervals during the fall when various amounts of moisture were in the soil. It was impossible to complete these experiments either year due to rather unusual weather conditions. The fall of 1919 was one of the driest on record and there was not enough moisture in the soil to cause good germination of wheat until late in October. Numerous plantings which were made, however, germinated in part where sufficient moisture was available. Whenever this early germination took place less smut developed than in wheat from seed which laid in the dust until after the rains began. Soil moisture determinations were made in a large number of fields in the fall of 1919 on farms in several counties of northern Idaho at the time when these fields were seeded. Although the data secured from this source were not by any means complete due to the lack of germination in early seeded fields, nevertheless, all the notes accumulated indicate that where wheat was sown after threshing had begun and after the soil had become infested by wind-blown bunt spores, the less the moisture in the soil at the time of germination, the less bunt developed in the crop of 1920.

One series of rod-row plantings was made on a piece of summer fallow ground which was especially well situated and which contained more moisture than the average soil. This series germinated fairly well and table number I gives the result of this experiment. Treated Jenkins Club wheat was used.

A Planet Jr. hand planter was used in making these rod-rows plantings and every effort was made to simulate field practices as much as possible.

The fall of 1920 was even less favorable for experiments of this nature. After September 10 there was no time in the fall when the average soil contained less than 15 per cent moisture. The ground was too wet for seeding during much of the time after that date.

TABLE I

*The effect of soil moisture and cultivation upon bunt infection.*

	Series I*	Series II*
	Percent of Smut	Percent of Smut
(a) Ground not cultivated before planting.....	8	3
(b) Ground cultivated before planting.....	10	4
(c) Soil smutted, ground not cultivated.....	50	8
(d) Soil smutted, ground cultivated.....	55	15
(e) Seed smutted before planting.....	75	30

\* Planted Sept. 1—Soil Moisture 16 per cent.

\*\* Planted Sept. 25—Soil Moisture 10 per cent.

## GREENHOUSE EXPERIMENTS

In order to test the effect of both moisture and temperature of the soil at planting time upon the amount of bunt in wheat, means were perfected for controlling both moisture and temperature of the soil within certain limits in greenhouse experiments. Three different ranges of temperatures were tried. The first, ranging from 9 to 12° C. was secured by means of a galvanized iron tank through which water was circulated. The second, ranging from 25 to 28° C. was secured by means of an electrically heated and controlled glass chamber. For the third room temperature which varied from 17 to 25° C. during the experiment was employed. Four lots of soil were used with moisture content as follows: No. 1, 13 to 14 per cent; No. 2, 16 to 17 per cent; No. 3, 18 to 23 per cent; and No. 4, 28 to 32 per cent.

The plants were grown in four-gallon jars. Bunt spores were added to the soil and Jenkins Club wheat, which had been mixed with viable bunt spores, was planted 1½ inches deep in each jar. The soil was kept at constant moisture content by covering the jars with empire cloth until the wheat had all germinated and emerged. Even in the series where the soil contained the least moisture, drops of water collected on the under side of the cloth and on the soil. It is the author's opinion that there was probably more germination of bunt spores in soil kept in a humid atmosphere than there would have been in a well aired soil.

Livingston porous cup soil irrigators were tried as a means of holding the soil at constant moisture content. These were not satisfactory as the writer was not successful in holding the moisture content of the soils used below 15 per cent.

TABLE II

*Effect of soil moisture and various soil temperatures upon infection of wheat with Tilletia tritici.*

Series No. 1.			
No.	Moisture	Temperature, degrees C.	Per cent Smut
1-1	14	9 to 12	40
2-1	17	9 to 12	71
3-1	22	9 to 12	90
4-1	32	9 to 12	19
Series No. 2			
1-2*	13	17 to 25	25
2-2.	16	17 to 25	20
3-2	18	17 to 25	21
4-2	32	17 to 25	0
Series No. 3.			
1-3	14	25 to 28	5
2-3	17	25 to 28	0
3-3	23	25 to 28	3
4-3	28	25 to 28	0

\* Water accidentally leaked into this jar.

Table number 2 indicates clearly the influence of both moisture and temperature of the soil on bunt infection. It will be noted that in No. 4 of each series where the soil approached a state of saturation at 28 to 32 per cent moisture little smut was developed (Fig. 2). The soil used in the greenhouse was a mixed soil with a moisture equivalent of 20.7. As shown above in connection with field experiments it was found that 100 per cent bunt developed in wheat where the soil at the time of germination of the seed contained 35 per cent moisture. This was in field soil, the moisture equivalent of which was 27.2. These results show the value of finding the moisture equivalent of each soil in order to obtain a physical constant for purposes of comparison.

Figure 3 illustrates the effect of high soil temperature with the different soil moistures; figure 4 the effect of low soil temperature with the various soil moistures, and figure 5, the effect of high soil moisture with the various soil temperatures.

#### LENGTH OF LIFE OF BUNT SPORES IN THE SOIL

Woolman (16) has shown that bunt spores which have lain over winter on the open ground in wheat heads at Pullman, Washington, may retain their power to infect in the spring. Heald and Woolman (6) conclude: First, that the infection power from separate spores is

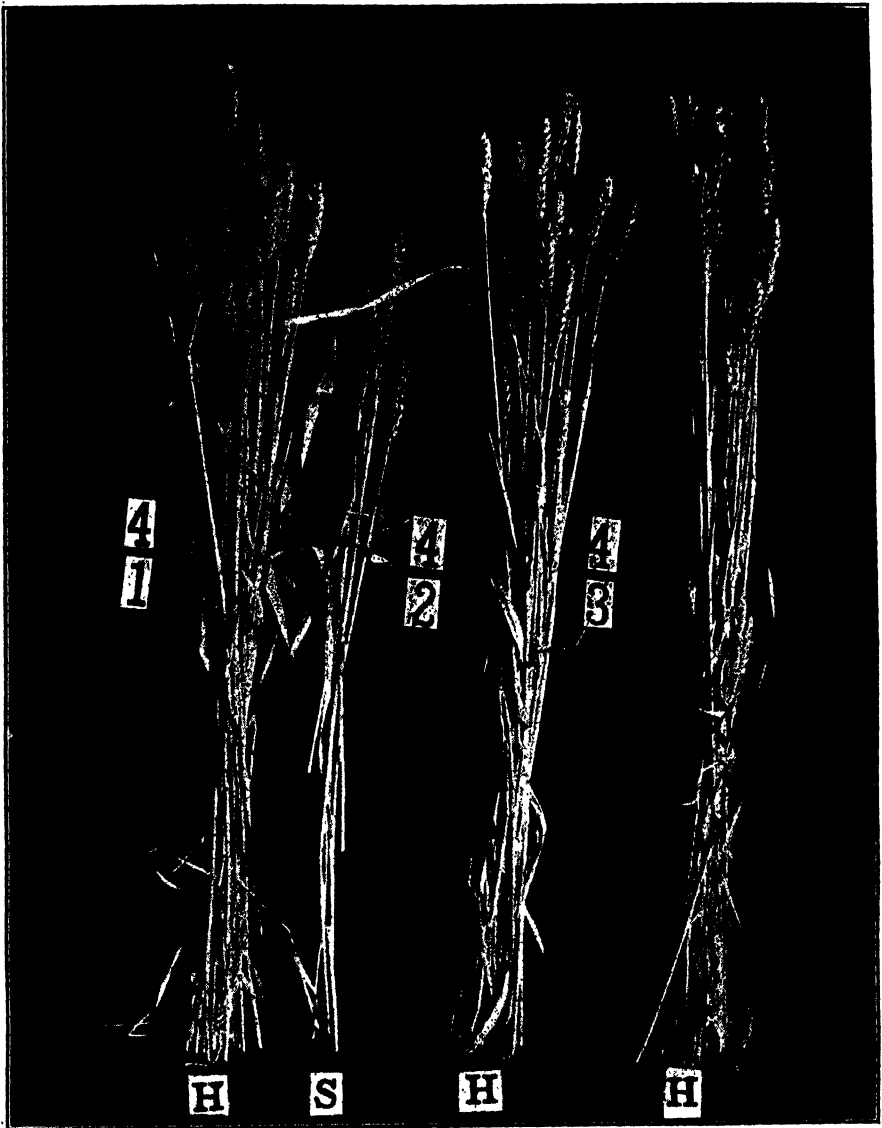


FIG. 2. Illustrating the effect of nearly saturated soil upon bunt infection.

Number	Percentage moisture	Temperature, degrees C.
4 /1	32	9 to 12
4 /2	32	17 to 25
4 /3	28	25 to 28

The bundles marked H contain healthy heads. Those marked S contain smutted heads.



FIG. 3. Illustrating the effect of high soil temperature with various amounts of moisture in the soil.

Number	Percentage moisture	Temperature, degrees C.
1 /3	14	25 to 28
2 /3	17	25 to 28
3 /3	23	25 to 28
4 /3	28	25 to 28

The bundles marked H contain healthy heads. Those marked S contain smutted heads.

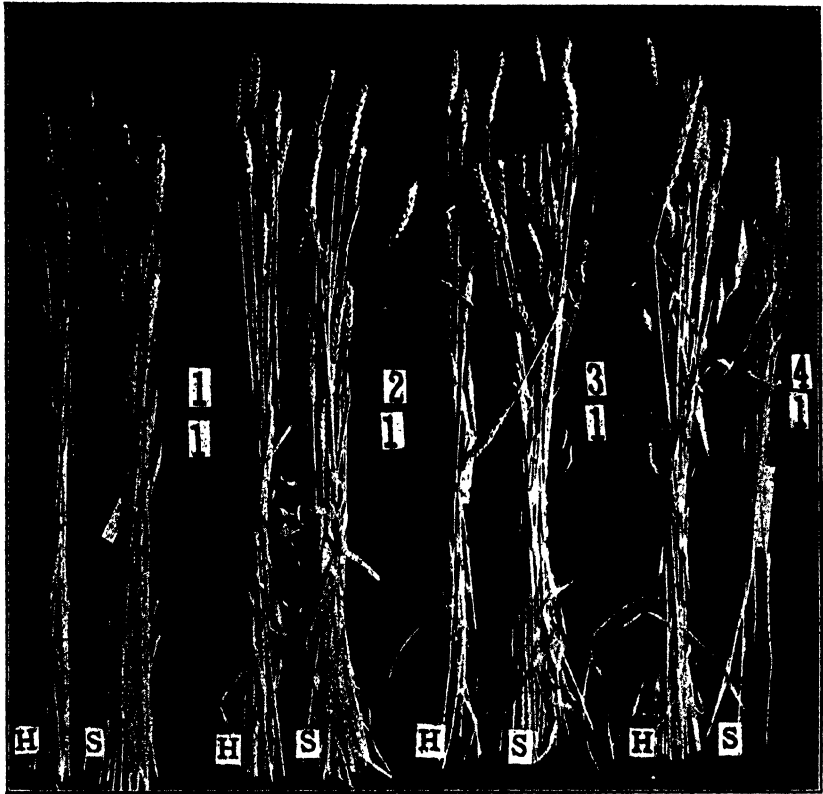


FIG. 4. Illustrating the effect of low soil temperature and various amounts of moisture in the soil.

Number	Percentage moisture	Temperature, degrees C.
1 /1	14	9 to 12
2 /1	17	9 to 12
3 /1	22	9 to 12
4 /1	32	9 to 12

The bundles marked H contain healthy heads. Those marked S contain smutty heads.





FIG. 5. Illustrating the effect of various temperatures with high moisture content in the soil.

Number	Percentage moisture	Temperature, degrees C.
3 /1	22	9 to 12
3 /2	18	17 to 25
3 /3	23	25 to 28

The bundles marked H contain healthy heads. Those marked S contain smutty heads.

limited to 2 or 3 months in moist soil, and, second, that the spores in unbroken balls may retain their vitality for one year or more under natural field conditions. Güssow (4) reports experiments which tend to show that bunt spores may survive very low temperatures, even remain viable after being frozen in a block of ice. If, however, they have started to germinate, freezing will destroy their power to continue growth.

In the fall of 1920 a series of experiments was started to determine, first, the length of time bunt spores in the soil will remain viable under various conditions of soil moisture, and, second, the effect of cultivation upon bunt infection from the soil. The ground was uniformly and thoroughly smutted September 4 by scattering bunt spores over the surface. The ground was then divided into 4 plots one rod square. Each plot was treated as follows: Plot No. 1 was watered thoroughly every 3 days and cultivated the day after watering. Plot No. 2 was watered thoroughly before the first planting and then cultivated before each later planting. To Plot No. 3 no water was applied and the ground was cultivated before each successive planting. To Plot No. 4, no water was applied and the ground was not cultivated.

Successive plantings were made in each plot September 4, 11, 24, and October 2. Jenkins Club wheat was used which had been treated with bluestone by the standard method, by dipping in a solution of 1 pound of bluestone to 5 gallons of water for 10 minutes.

Soil moisture tests were made in each plot at each planting and after each watering or rainfall. Water was applied to the plots which were watered the day before the wheat was planted. In each case where watering was done the soil samples were not taken for several hours after the water was applied so as to allow for distribution of the moisture through the soil. The temperature of the soil was also taken at these intervals. Table No. 3 gives the results of this experiment.

TABLE 3

*Effect of soil moisture and cultivation upon the length of life of bunt in the soil.*

		Plot 1. Wet continually. Cultivated.		Plot 2. Wet at beginning. Cultivated.		Plot 3. Dry. Cultivated.		Plot 4. Dry. Not cultivated.	
		Mois- ture	Per cent Smut	Mois- ture	Per cent Smut	Mois- ture	Per cent Smut	Mois- ture	Per cent Smut
Sept.	4	20	30	22	35	10	19	10	23
	8	22	—	20	—	10	—	10	—
	11	24	10	23*	35	17	14	18	8½
	14	28	—	20	—	18	—	18	—
	24	22	4½	21	13	20	6	20	5
Oct.	2	20	2	20	0	18	4½	19	3

Rainfall on September 11 amounting to over 0.25 inch interfered somewhat with the experiment. Doubtless the results would have been much more striking if no rain had fallen between the successive plantings. The numbers at the top of the table refer to the 4 plots listed above, the dates to the time of planting, and to the time moisture determinations were made. The moisture content taken on the dates indicated is given as the percentage of moisture in the soil and was computed on a dry basis as in previous experiments. The percentage of bunt was figured on the basis of the number of infected plants in the resulting crop. Soil temperatures varied from 50° to 70° F. during the period from September 16 to October 2.

#### SUMMARY

The control of bunt or stinking smut is one of the most difficult problems in the Pacific Northwest where the soil becomes infested with the spores of *Tilletia tritici* blown by the wind from threshing machines and combines.

It has been known for a number of years that soil contamination by wind-blown spores is almost universal in the Palouse region. More recently it has been shown that this condition exists in other regions as well.

Field observations in northern Idaho have shown that there is a very definite relation between the amount of moisture in the soil at seeding time and the amount of bunt which will occur in the resulting crop of wheat. Numerous cases have been observed where part of a field sown in dry soil produced a crop of wheat containing only a trace of bunt and where the rest of the field sown with the same seed after it had rained produced a very smutty crop.

Wheat was grown at Moscow, Idaho, in 6 soil containers filled with bunt infested soil varying in moisture content from 8 to 32 per cent at the time of planting and with a moisture equivalent of 27.2. The amount of bunt which developed in these containers varied from none to 100 per cent. The per cent of bunt increased progressively as the percentage of soil moisture increased. This experiment was repeated at Nez Perce, Idaho, with similar results.

Numerous field observations and field plot experiments tend to show that during the time when the soil is infested with viable spores of *Tilletia tritici*, the amount of moisture in the soil at planting time has a direct influence upon the amount of bunt in the resulting wheat crop.

Greenhouse experiments under controlled conditions have shown that low soil temperatures and a fairly high percentage of moisture in the

soil are both conducive to stinking smut infection. The highest percentage of infection was secured at temperatures ranging from 9° to 12° C. (48° to 54° F.) and in soil containing 22 per cent moisture and with a moisture equivalent of 20.7.

Under greenhouse conditions an exceedingly high percentage of moisture in the soil seemed to inhibit infection.

Some bunt infection was secured when the soil was held at a temperature of 25–28° C. (77° to 82° F.) during the germination of wheat.

Preliminary experiments appear to indicate that spores of *Tilletia tritici* in the soil lose their power to infect rather rapidly when the soil is moist and is cultivated frequently. Very little infection took place from spores which had been in the soil one month under the above conditions.

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# PHYTOPATHOLOGY

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## THE RELATION OF TEMPERATURE AND HYDROGEN-ION CONCENTRATION TO UREDINIOSPORE GERMINATION OF BIOLOGIC FORMS OF STEM RUST OF WHEAT<sup>1</sup>

C. R. HURSH

WITH SEVEN FIGURES IN THE TEXT

Although it has been shown that there are numerous biologic forms of *Puccinia graminis* on wheat, which may be differentiated by their parasitic action on certain wheat varieties, the physiologic basis for these differences has never been satisfactorily explained. It has been suggested that their parasitic range may be limited by extremely narrow nutritional requirements furnished by some wheat varieties, but not by others. There has been considerable difficulty, however, in attempting to explain the parasitic action of the biologic forms of the wheat stem rust by this hypothesis. Since the physiology of these forms apart from their hosts has not been studied, and considering the fact that most species and varieties of fungi are known to be quite different physiologically, this study was begun in order to determine whether physiological differences could be demonstrated for the biologic forms of the stem rust of wheat without the aid of differential host varieties.

As the rusts have not been cultivated in artificial media these studies were necessarily confined to spore germination, and while spore germination was not the most desirable attack on such a complex problem, it was nevertheless the only one available. Fortunately it has proved itself to be admirably suited to the problem at hand. The urediniospores were selected as the spore forms most suitable for germination studies.

On account of the apparent regional distribution of the biologic forms, which conceivably could be due to temperature relations, the effect of

<sup>1</sup> Co-operative Investigation between the Bureau of Plant Industry of the United States Department of Agriculture and the Agricultural Experiment Station of the University of Minnesota.

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temperature on spore germination was studied. As the behavior of certain species of fungi, in relation to the reaction of a given substrate is known to be specific and characteristic, a study was made of the range of tolerance of biologic forms to hydrogen-ion concentration. Such factors as osmotic concentration of the substrate, influence of specific substances, and various surface-action phenomena also appeared to be worthy of consideration. The experiments reported in this paper, however, deal only with the effect of temperature and hydrogen-ion concentration.

Age and maturity of the urediniospores of wheat stem rust, as well as conditions under which they are developed, and even the conditions to which they are subjected after maturity, all tend to influence germination. Hence in this study it was necessary to eliminate all factors except the one of difference in biologic form alone. This was attempted by using urediniospores of the same age, which were developed under similar greenhouse conditions on Little Club wheat, a host equally susceptible to the biologic forms studied. By using such precautions it was possible to secure constant and uniform results. As a check on the constancy of behavior which could be expected under the conditions of the experiment, germination studies were made on certain forms for a period of over twelve months with the same uniform results throughout the period. During the time the forms passed through at least twelve "urediniospore generations."

#### MATERIALS AND METHODS

From a number of biologic forms which were studied in preliminary tests, two were selected for further study because of their wide parasitic difference on certain host varieties. One of the forms selected was collected by Professor W. W. Mackie, at El Centro in the Imperial Valley of California, and sent to the Minnesota laboratory for identification. It attacks Marquis, Kanred, Kota, Arnautka, Mindum, Speltz Marz, Kubanka, Acme, and Einkorn quite readily, but it does not produce any infection on White Spring Emmer (Minn. 1165) other than a flecking. The other form was collected by Dr. J. Dufrenoy, at Barèges, France. Of the wheat varieties mentioned, it attacks only Kubanka and Acme in a similar manner to the El Centro strain. On the other hand, it fails entirely to attack either Kanred or Kota, and produces only flecks upon Arnautka and Einkorn. It attacks Marquis, Mindum, and Speltz Marz very weakly, and in contrast to the form from California produces a heavy infection upon Emmer. While such extreme difference in parasitism between two biologic forms is not the rule, it

is quite characteristic of what may be expected in different host reactions with biologic forms of the wheat stem rust. Table 1 shows the parasitic behavior of these two forms.

The temperatures used in these experiments were 10, 20, and 30° C. These were maintained by icebox and control incubators. There was a plus and minus variation of one degree for the two lower temperatures. At 30° C there was practically no variation. In every case germination was studied in a series of solutions which represented a range of hydrogen-ion concentration, thus giving an opportunity to observe the combined influence of temperature and hydrogen-ion concentration. Monobasic acid potassium phosphate was used as a buffer and hydrochloric acid or sodium hydroxide was added. The hydrogen-ion concentration was determined with the potentiometer. Many solutions were actually made up to be at convenient intervals on the pH scale and only those were used which gave a representative range. The method of making up these solutions is given in table 2.

TABLE 1

*The effect of Biologic Forms XI and XXVII on differential hosts<sup>1</sup>*

Host	Degree of infection	
	Biologic form XI	Biologic form XXVII
<i>Triticum vulgare</i>		
Marquis C. I. 3641.....	Very heavy	Very weak
Kanred C. I. 5146.....	do	No infection
Kota C. I. 5878.....	do	do
<i>Triticum durum</i>		
Arnautka C. I. 4072.....	do	Flecks only
Mindum C. I. 5296.....	do	Very weak
Speltz Marz C. I. 6236.....	do	do
Kubanka C. I. 2094.....	do	Very heavy
Acme C. I. 5284.....	do	do
<i>Triticum monococcum</i>		
Einkorn C. I. 2433.....	do	Flecks only
<i>Triticum dicoccum</i>		
Emmer C. I. 3686.....	Flecks only	Very heavy
<i>Triticum compactum</i>		
Little Club C. I. 4066	Very heavy	do

<sup>1</sup> These host relations were determined by Dr. E. C. Stakman and Mr. M. N. Levine, Co-operative project between the Office of Cereal Investigations, United States Department of Agriculture and the Division of Plant Pathology, University of Minnesota. The forms had been cultured in the greenhouse at least one year previous to this study.



TABLE 2

Preparation of solutions used for the study of the influence of hydrogen-ion concentration on germination of spores of biologic forms

on germination of spores of biologic forms					pH	
1	50 cc	$\frac{M}{5}$ $\text{KH}_2\text{PO}_4$	+	$\frac{M}{5}$ $\text{HCl}$	+ water to 200 cc	2.5
2	do	+	do	+	do	3.2
3	do	+	do	+	do	4.2
4	do	+	$\frac{M}{5}$ $\text{NaOH}$	+	do	5.2
5	do	+	do	+	do	6.0
6	do	+	"	+	do	7.0
7	do	+	"	+	do	8.0

In making the germination tests 3 cc. of solution were placed in a clean Syracuse dish, and the spores were placed on the surface of the solution. The dishes were then stacked and incubated for twelve hours. This method was checked against the ordinary hanging drop method and found to be superior. Hundreds of spores can quickly be observed on the surface of the solution in the Syracuse dish in contrast to the much smaller number of spores in the hanging drop. Furthermore the errors introduced through evaporation, distillation of drops, and irregular germination at the margin of drops due to surface action, are obviated by the use of the Syracuse dish.

TABLE 3

The relation of temperature and hydrogen-ion concentration to two biologic forms of *Puccinia graminis tritici*

Hydrogen-ion concentration-values of pH								Temperature
	2.5	3.2	4.2	5.2	6.0	7.0	8.0	Totals
Form								
XXVII			Per cent germination					
10 C.....	1	6	34	32	66	15	2	156
20 C.....	6	14	66	91	84	54	8	323
30 C.....	1	3	14	15	33	8	2	72
Average.....	3	8	35	46	61	26	4	
Form								
XI								
10 C.....	2	29	92	88	91	89	0	391
20 C.....	3	32	96	88	94	78	5	396
30 C.....	2	7	85	75	87	25	7	288
Average.....	2	23	91	84	91	64	4	

## RESULTS

The data used in making the accompanying graphs were secured from

three complete series observed on different dates, and from spores of succeeding generations. These data are summarized in table 3. Each figure given as a percentage germination represents a count of 600 spores, being the average of three percentages based on the count of 200 spores for each temperature and hydrogen-ion concentration of three separate series.

Figure 1 shows a comparison of the percentages of germination of the spores of the two forms in a hydrogen-ion concentration range at 10° C. The spores of Form XI germinate in a much wider range of hydrogen-ion concentrations than do the spores of Form XXVII, and

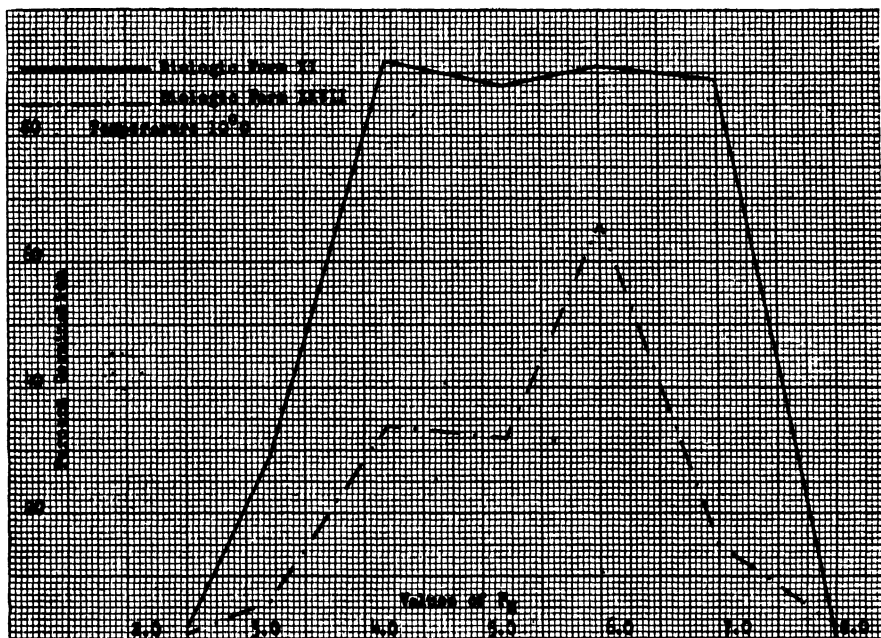


FIG. 1. Relation of hydrogen-ion concentration to urediniospore germination of biologic forms. Temperature 10° C.

the percentage of germination also is consistently higher. Figure 2 shows that at 20° C there is less difference in the behavior of the spores of the two forms so far as maximum germination is concerned, but the spores of Form XI still are more tolerant to the hydrogen-ion extremes. As shown by figure 3, the tolerance of the spores of Form XI decreased at 30° C, whereas the germination of the spores of Form XXVII is decidedly inhibited throughout by this higher temperature. Figure 4 permits an easy comparison of the effect of temperature on the behavior of Form XI in the hydrogen-ion concentration range, and figure 5 shows

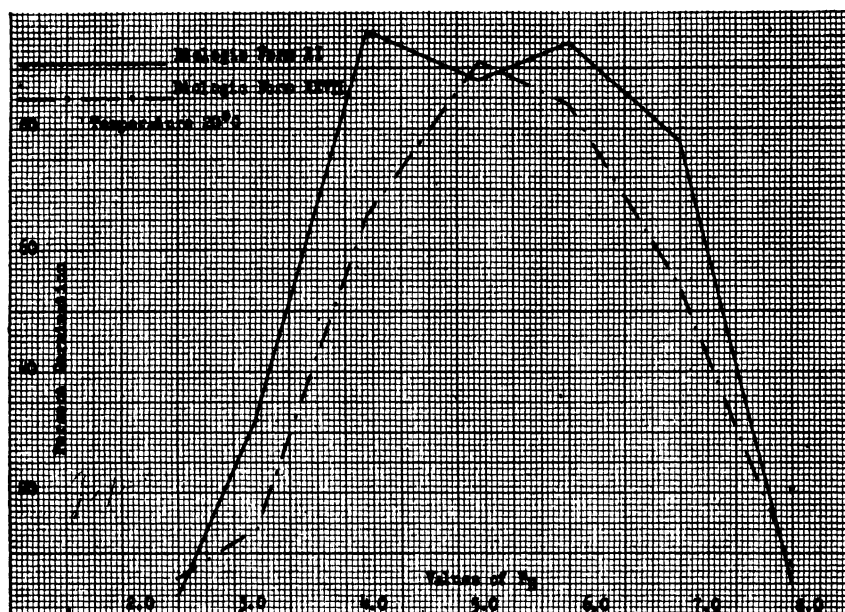


FIG. 2. Relation of hydrogen-ion concentration to urediniospore germination of biologic forms. Temperature 20° C.

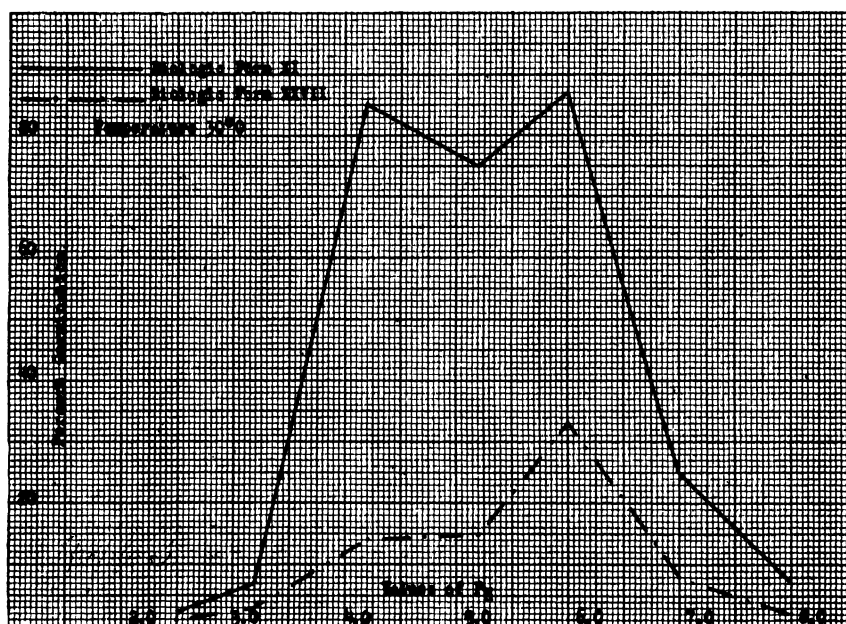


FIG. 3. Relation of hydrogen-ion concentration to urediniospore germination of biologic forms. Temperature 30° C.

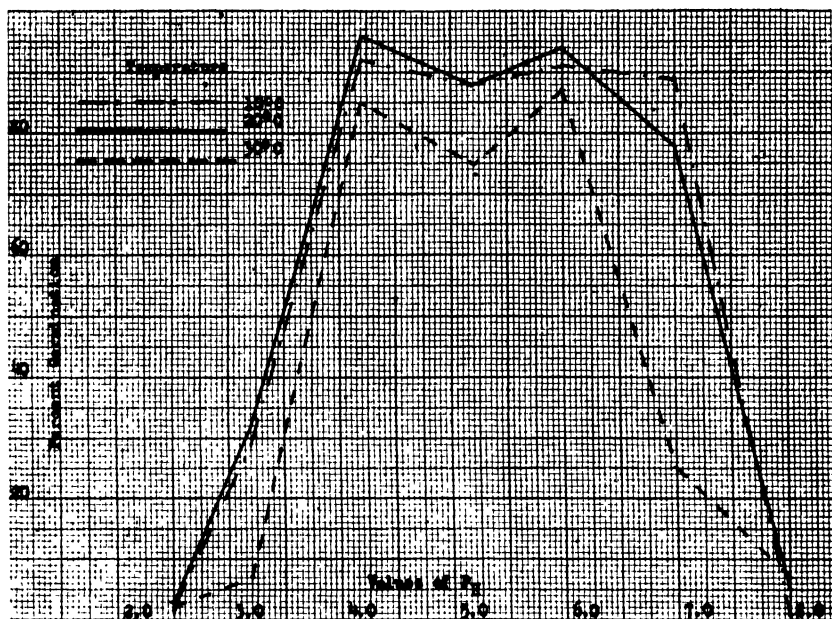


FIG. 4. Relation of temperature and hydrogen-ion concentration to urediniospore germination of Biologic Form XI.

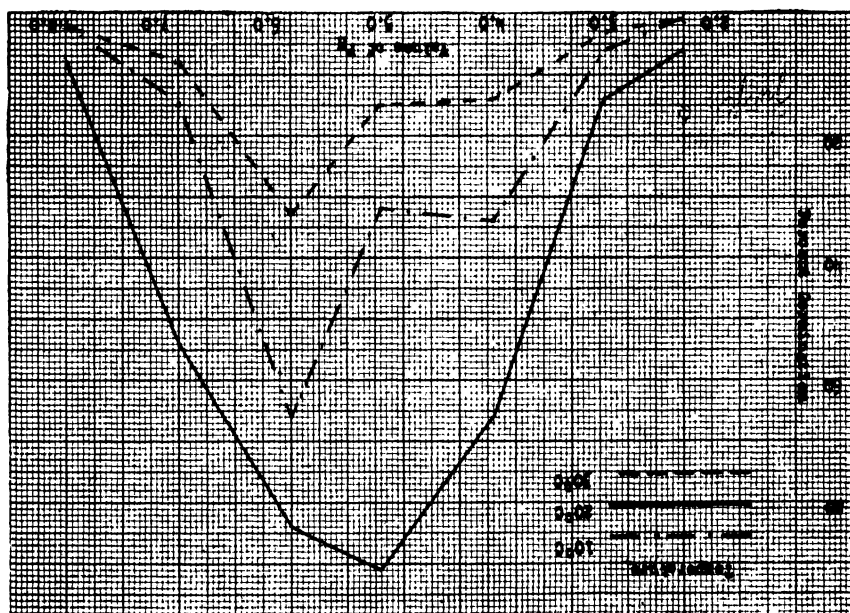


FIG. 5. Relation of temperature and hydrogen-ion concentration to urediniospore germination of Biologic Form XXVII.

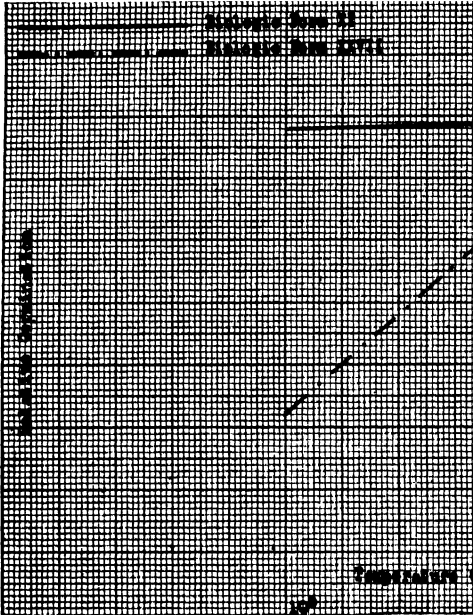


FIG. 6. Relation of temperature to urediniospore germination of biologic forms.

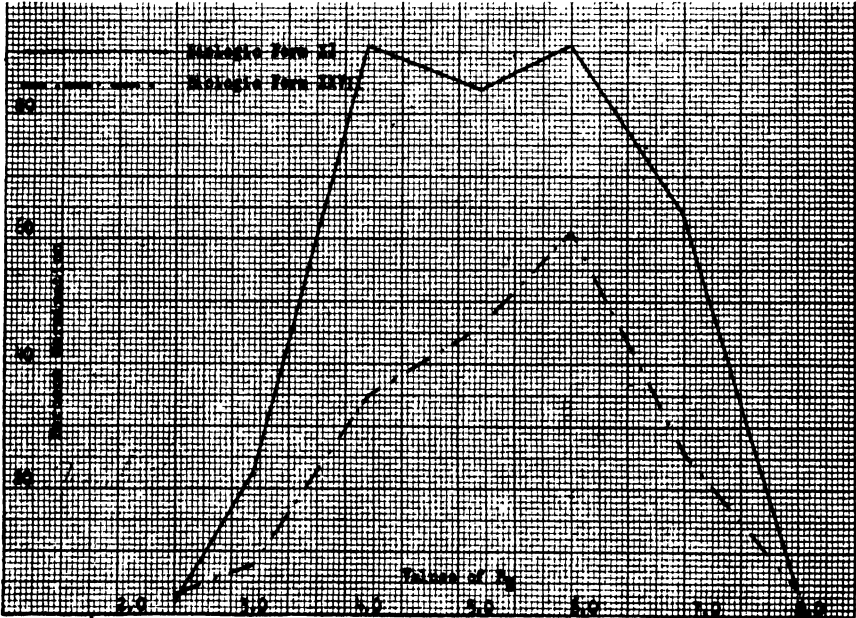


FIG. 7. Relation of hydrogen-ion concentration to urediniospore germination of biologic forms.

the analagous data for Form XXVII. Figure 6 summarizes the data secured for the two forms, considering temperature only, and in a similar way figure 7 summarizes the influence of the hydrogen-ion concentration of the solution.

In the data presented there appears to be a positive correlation between limited host range and restricted tolerance of the germinating urediniospores to temperature and hydrogen-ion concentration. Whether this correlation may be of significance in a study of resistant hosts is a problem for further investigation. In general these data show that the genetic constitution of two biologic forms of stem rust of wheat is essentially different in each form. It would appear that biologic forms differ from one another in their capacity for physiologic behavior and this in turn determines their action on differential hosts.

#### SUMMARY

In summarizing this preliminary report the following facts seem to have been established.

1. Two biologic forms of *Puccinia graminis* of wheat, differing in their parasitic behavior, show a considerable difference in germination response to temperature and hydrogen-ion concentration.
2. The form more limited in its host range is also more restricted in tolerance of extremes of hydrogen-ion concentration and temperature.
3. The differentiation of biologic forms is not entirely dependent on their parasitic behavior on certain host plants. At least some biologic forms apparently possess individual physiologic characteristics demonstrable by physical and chemical studies, and these characteristics alone may be sufficient to establish them as definite taxonomic entities.

# NOTES ON CHEMICAL INJURIES TO THE EASTERN WHITE PINE (*PINUS STROBUS* L.)

WALTER H. SNELL AND NATHANIEL O. HOWARD

WITH PLATE XXIV AND TWO FIGURES IN THE TEXT

## INTRODUCTION

Injuries to the eastern white pine (*Pinus strobus* L.) are often very conspicuous because of the contrast in color between the yellow-green, red or brown-red needles of injured portions and the bright green of the normal foliage. As one travels through white pine country he notices many such discolorations which challenge his interest and demand explanation. Outside of the single branch injuries which may be due to mechanical causes, to the white pine blister rust fungus (*Cronartium ribicola* Fischer), or to certain insects such as the pine weevil (*Pissodes strobi* Peck), and pine bark louse (*Chermes pinicorticis* Fitch), there are many troubles which affect the entire tree. Of these general disturbances indicated by the conspicuous deviations from the normal fresh green color of the foliage and popularly called "blights," there may be mentioned the following which in each case can be traced to some definite cause: 1, a bronzed appearance of the foliage of entire trees over large areas due to the annual dying of the older needles; 2, the common spring "blight" of the foliage of entire trees or of portions of trees, commonly on the side of the prevailing winds and sometimes observable over extensive areas, caused by winter drying; 3, the death of tops or of other comparatively large portions of good sized trees, or of entire trees in the case of the smaller ones, noticeable in more restricted areas and caused by *Cronartium ribicola* Fischer; 4, the death of numerous trees in more or less localized areas in pine lots due to the root-rotting fungus, *Armillaria mellea* (Vahl.) Quel.; 5, the occasional death of trees in swampy regions due to drowning, i. e. lack of proper aeration of the roots. In addition to these one often observes trees with diffuse red-brown foliage, sometimes alone, often in groups and either dead or still alive, the condition of which defies a ready diagnosis. This paper gives observations made during the summer of 1921, upon the reddening of white pines due to two entirely different causes but both of a chemical nature.

## INJURY CAUSED BY TOXIC FUMES

The first type which was observed in eastern Massachusetts during the early part of August was characterized by a reddening of the foliage of white pines *en masse* over an area perhaps  $\frac{1}{2}$  mile long by  $\frac{1}{4}$  mile wide at the broadest point and appearing even at a distance of two or



FIG. 1. Branch of white pine showing the effect of acid fumes. The lighter colored portions of the needles were dead and reddish brown, while the dark portions were still green and living.

three miles as a rich red blotch in the general landscape. At a distance of a few yards the needles seemed to be entirely dead, so evenly diffused was the red color of the foliage. A close examination of the twigs, however, revealed the fact that all of the needles were not dead and that the trees were still alive (Fig. 1). The younger needles appeared to



have suffered most, though nearly all were discolored to some extent. Further examination of the trees in this area disclosed the fact that not only the pines, but also many of the deciduous trees, i. e., black oak (*Quercus velutina* Lam.), white oak (*Quercus alba* L.), swamp white oak (*Quercus bicolor* Willd.), gray birch (*Petula populifolia* Marsh.),

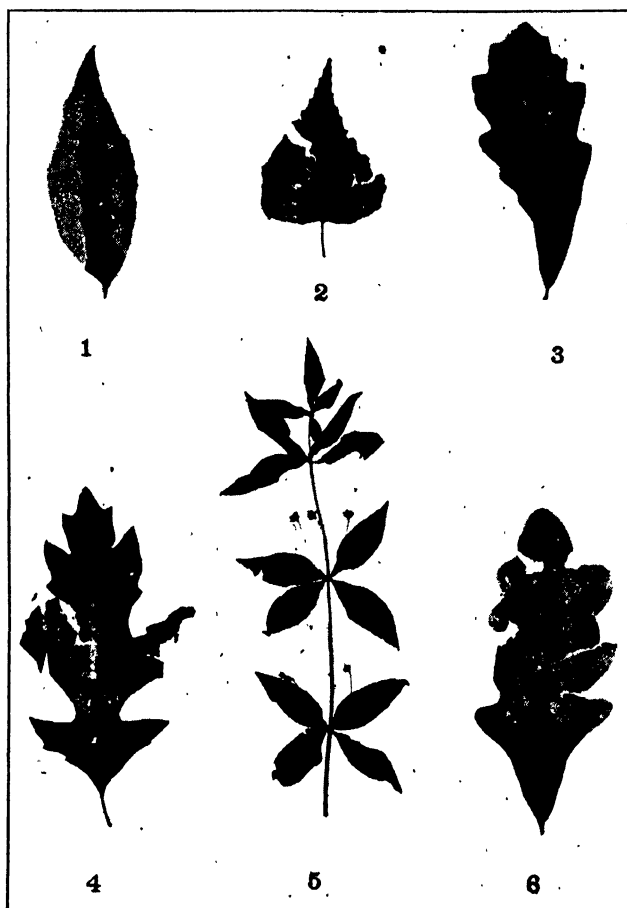


FIG. 2. Various leaves from the discolored area showing the effects of the acid fumes in the marginal blanching. 1, Black cherry (*Prunus serotina* Ehrb.), 2, Gray birch (*Betula populifolia* Marsh), 3, Swamp white oak (*Quercus bicolor* Willd.), 4, Black oak (*Quercus velutina* Lam.), 5, Whorled loosestrife (*Lysimachia quadrifolia* L.), 6, White oak (*Quercus alba* L.).

wild black cherry (*Prunus serotina* Ehrb.), and even such plants as poison ivy (*Rhus toxicodendron* L.), whorled loosestrife (*Lysimachia quadrifolia* L.), and brake (*Pteris aquilina* L.) bore evidence of injury. In the majority of these cases the damage was manifest in the marginal browning or blanching of the leaves (Fig. 2). It is of interest to note

that hickory (*Carya sp.*), elm (*Ulmus americana* L.), and blue spruce (*Picea pungens* Engelm), however, were apparently unaffected. The limits of the diseased area were well defined, this being particularly true of the western border. Norway spruces (*Picea excelsa* Link.) lying just within the borderline bore many reddened leaves, while those but a few feet further to the west and apparently outside of the zone were perfectly normal.

According to certain persons living or working in the immediate vicinity, this type of injury has appeared in identically the same spot during the early part of the past two summers. In each instance it was noted that the majority of the trees recovered and developed normal foliage. Occasional trees, however, succumbed. Another lot of pines lying approximately  $\frac{1}{2}$  mile to the northeast had been so badly injured during previous years that it was found necessary to fell them.

Because of the strikingly localized character of the affection, one naturally sought the cause in the immediate vicinity. Three possible sources only were found: i. e., a railroad paralleling the diseased area on the east, a power plant three quarters of a mile to the northeast, and a brick kiln but a few hundred yards to the north. After a thorough investigation the first was eliminated, for it was observed that trees outside of the discolored area, yet between it and the tracks were unaffected. The power plant seemed to be too far removed to be considered responsible for the injury, though a careful study of the drift of smoke issuing from the tall stack was made. Suspicion pointed strongly toward the fumes from the brick kiln. This kiln is located at a point 300 yards north of the discolored area and perhaps 120 yards from the nearest pine. Furthermore, the affected trees lay in a more or less fan-shaped area with the apex pointing directly toward the kiln to the north. Finally, it was observed that the intensity of the discoloration varied with the distance from the kiln, it being most conspicuous at the apex of the fan-shaped area and gradually diminishing towards the limits farthest from the kiln. If it could be proved that toxic fumes had emanated from the kilns within the preceding few weeks and that prevailing winds during that time had favored a drift of these fumes toward the pines, an explanation of the phenomenon would be found. Inquiries were made at the office of the brick yard and records of kiln burnings were studied. It was learned that the burnings lasted for 9 days each with quiescent intervals of 2-9 days during which the fires were extinguished and the bricks removed. It is the custom to use bituminous coal in the arches of the kilns during each burning, and usually during the last three or four days. This material is placed upon the burning wood, the customary fuel, in order "to draw the fire." It

is probable that acid fumes resulting from the combustion of coal of relatively high sulphur content were carried to the pines by north, northwest or northeast winds and brought about the phenomenon here described.

Records of the local station of the weather bureau for the two months immediately preceding were examined. Table 1 shows the relation of the time of the appearance of the discoloration to the periods of kiln burning and to certain meteorological data.

TABLE 1

*The relation of the time when the discoloration of the pines was first observed to the periods of kiln burning and to certain meteorological data*

Dates of kiln burning	Direction of wind	Temperature degrees F	Relative humidity percentage	Precipitation In.	Condition of pines
June					
8	SW-S	68	86	0.	Good
9	SW-S	68	85	0.	ditto
10	SW-S	67	84	0.	"
11	SW-S	67	87	0.	"
12	W-W	80	86	0.	"
13	SW-SW	69	87	0.	"
14	NW-N	65	84	0.	"
15	NW-N	67	84-90	.03	"
16	NW	66	84-86	0.	"
17	NW-SW	68	84-86	0.	"
28	SW-NE	73	95-96	.01	Good
29	SW-NE	72	91-90	.18	ditto
30	N	63	100	3.00	"
July					
1	N-NE	61	100	.37	"
2	N-NW	61	100	1.13	"
3	SW-E	74	86-88	.03	"
4	NW-NE	82	88	1.22	"
5	NE-E	72	85-88	0.	"
14	SW	84	86-88	0.	Good
15	SW-NE	76	86-100	.08	"
16	NE	68	85-86	.24	Reddening first reported
17	SW-S	74	87-86	0.	" increasing
18	SW	73	86-87	0.	ditto
19	SW	74	81-87	0.	"
20	NW-N	77	91-94	.33	"
21	NE	65	94-86	.07	"
22	NW-NE	69	85-86	0.	"
23	NE	70	85-87	0.	Reddening pronounced

It will be observed that during the latter part of each period of kiln burning, i. e. during those days when coal was being used as part fuel, the wind blew from a general northerly direction. This would carry the gaseous products of combustion toward the pines. These data then harmonize fairly well in the support of the hypothesis that such fumes arising from the arches of the kilns were directly responsible for the injury, especially when taken into consideration with the following facts noted previously:

- 1, the distinctly localized character of the injury;
- 2, the striking fan shape of the area, with the apex pointing toward the kiln;
- 3, the gradual decrease in the intensity of the discoloration toward the limits farthest from the kiln;
- 4, the sharp lateral delimitation of the area;
- 5, the injury to broad-leaf trees, herbaceous plants and ferns as well as to the conifers, within the same area.

Abundant evidence can be found to support the statement that sulphur dioxide will cause the discoloration of pines. It is difficult to state however, without experimental data the exact time of the initial attack in this particular instance. In all probability the effect was cumulative.

#### INJURY DUE TO A CHEMICAL SUBSTANCE IN THE SOIL

The second case of chemical injury was the reddening of the foliage and death of individual pine trees along a roadside in New Hampshire (Pl. XXIV). Trees of all sizes were affected. Those under 6 inches in diameter were killed and the mature pines either had large portions of the crown entirely killed or suffered the killing of parts of the needles more or less generally. The redness of the needles was hardly distinguishable from that resulting from fumes and referred to above. The cause was found to be a very simple one. Along this particular gravel road, calcium chloride, a hygroscopic salt, had been applied to the surface to act as a binder and thus prevent dust. This was the only treatment which the road has received for several years. Preparatory to the application of the calcium chloride, the metal containers had been distributed along the roadside at intervals and were placed in the majority of cases under pine trees. These barrels were not tight because of rough handling and the chemical either fell out directly in the dry condition or was dissolved out by the water which it had absorbed from the air. The barrels remained by the roadside for about a month (May 15 to June 15, roughly) during a period when the rainfall was very slight.

It would seem, then, that little of the salt could have been dissolved by other than the absorbed water. However, a sufficient amount of it seeped into the soil and caused the damage stated.

That the calcium chloride was the cause of the damage appeared evident inasmuch as every tree under which barrels had been placed was affected. Trees with barrels near the trunks were killed and those with barrels at a distance from the trunk yet within the limits of the root system suffered partially (Pl. XXIV). Other pines along the road for miles were in the best of condition.

The pines were not the only trees injured, for both birches and elms under which barrels of calcium chloride had been stored, suffered more or less severely. The injury to the deciduous trees consisted of the marginal drying and browning of leaves, together with a pronounced upward curling of the leaves and partial defoliation of the upper branches in the case of the elm. No killing of these trees was noted however. As in the case of the pines those deciduous trees with no barrels of calcium chloride at the base were in a healthy condition.

It may be added that common salt used upon roadways and walks for a similar purpose has been known to affect bordering trees, particularly maples, in much the same manner.

#### SUMMARY

This paper gives observations made during the summer of 1921, upon the reddening of the white pine due to two entirely different causes, but both of a chemical nature

1. A very conspicuous reddening of white pines in a limited area, perhaps  $\frac{1}{2}$  by  $\frac{1}{4}$  mile was observed in eastern Massachusetts. This injury apparently was caused by acid fumes emanating from the arches of a near-by brick kiln, wherein coal was occasionally used as fuel.

2. The reddening of pines along a road in New Hampshire was observed. This was found to be due to calcium chloride which had leached from barrels placed under pines by the roadside.

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## INJURIES TO WHITE PINE

Pine trees with discolored foliage caused by calcium chloride which seeped into the soil from barrels placed beneath the trees. The smaller one is entirely dead and the nearer portions of the larger tree above the smaller one may be seen to be dead.



TORULA LIGNIPERDA (WILLK.) SACC.  
A HYPHOMYCETE OCCURRING IN WOOD TISSUE

PAUL V. SIGGERS

WITH PLATE XXV

Late in 1919 there were received at the Forest Products Laboratory, Madison, Wisconsin, a shipment of white ash (*Fraxinus americana* L.) logs from Graham County, Tennessee and a shipment of yellow poplar (*Liriodendron tulipifera* L.) logs from east central Kentucky. After remaining a few weeks in a well-ventilated pile, the logs were cut into standard sizes for use in standard timber tests. When these tests were conducted the variation in strength values obtained, as compared with the values which would be expected in normal white ash and yellow poplar suggested an examination of the test pieces for evidence of fungous infection.

The test pieces had been selected carefully and would ordinarily have been considered sound, but examination showed dark catenulate spores scattered deeply in the wood of both species.<sup>1</sup> The spores were similar in both cases. Further study showed that the fungus was present in the heartwood, sapwood, and throughout the length of the white ash logs. In the ash test pieces the presence of the fungus was correlated with dark tan streaks which had a tendency to follow along the same group of annual rings. In cross section the discoloration appeared in more or less irregular zones. In the case of the yellow poplar the fungus was not so widely distributed as it was in the white ash, but in all cases where the wood showed a reddish pink tinge, the dark spores could be found easily. The color variation normally present in yellow poplar, from the greenish yellow, so characteristic of the wood, through the various darker colors, tended to mask any early stages of color change due to the fungus.

A number of transfers were made to agar in test tubes from the streaks in several blocks of yellow poplar. In two tubes out of thirty a dark slow growing fungus developed. All of the other twenty-eight tubes remained sterile. An examination of the fungus both in the cultures and in the wood showed the two to be identical.

<sup>1</sup> Miss Gertrude Griffin of the Forest Products Laboratory made the original microscopic examination, brought the fungus to the writer's attention and kindly translated a portion of Willkomm's "Die Mikroskopischen Feinde des Waldes."

The writer wishes to acknowledge also help received from R. H. Colley and E. E. Hubert in the preparation of the manuscript and photomicrographs.



A few weeks later the writer's attention was directed to a similarity between the fungus in the tube cultures and one noted by von Schrenk (8) as occurring, among others, in the wood known as "pecky" cypress. He called it *Xenodochus ligniperda*. The original description of *Xenodochus ligniperda* was published by Willkomm (9). He reported having observed spores of this fungus in spruce, fir, oak, buckthorn (*Rhamnus cathartica* L.), and french briar (*Erica arborea* L.). He believed the organism was responsible for what he called "red rot"; but, in the light of present day knowledge of the subject of wood decay, Willkomm's "red rot" was probably due to one of the higher fungi. He associated *Rhyncomyces violaceus* with *Xenodochus ligniperda* and asserted that the former had its origin in an alleged motile stage of the latter. He considered that the fungus was also related to Th. Hartig's *Nyctomyces fuscus*—a sterile form. Willkomm's drawings are very good, but the variety of fungi described forces the conclusion that he was dealing collectively with a large number of organisms. It is unfortunate that the technique of fungus isolation had not been developed at the time Willkomm did this work. R. Hartig (3) considered the fungus a Pyrenomycete. He often observed it in the roots of trees in association with *Armillaria mellea* and concluded that the organism was apparently able to penetrate cell walls. Saccardo (5) listed the fungus as *Torula ligniperda*, and as such it is referred to in this article. Sorauer (7) reviewed Willkomm's work several years later but made no original observations.

Members of the *Torula* group have been known for a long time as wood-inhabiting fungi. They have been noted as the cause of sooty or blackish discolorations on dead branches of woody and herbaceous plants and on pine timber which had been exposed to the weather. A few years ago, Miss Smith (6) isolated from partially decayed house timbers several Hyphomycetes, including *Torula* and *Haplographium*. She concluded that favorable conditions of moisture made possible the development of these forms in wood, and that under such conditions they accounted for its slow decay.

*Torula ligniperda* was found in red gum in 1911 by Dr. Eloise Gerry. This is the first record, so far as can be determined, of the appearance of the fungus in wood specimens examined at the Forest Products Laboratory. Since this study was begun, it has been found by E. E. Hubert forming dark bands running longitudinally with the grain of the wood in eastern hemlock (*Tsuga canadensis* Carr.), usually on the outer edge of the region of incipient decay of a brown cubical rot. It has also been found in basswood veneer and in maple furniture stock.

Several tests were started to determine if possible how the power of

this fungus to attack wood compared with that of Basidiomycetes. Small cubes of sapwood and heartwood of yellow poplar, none of which exceeded a cubic centimeter in volume and which had previously been immersed in water, were placed over moist cotton in large test tubes. They were then sterilized without pressure. Inoculation by transfer from pure cultures of the fungus was made March 26, 1920. Sixteen tubes were prepared; fourteen were inoculated and two were held as controls. The wood blocks were under observation for six months and the progress of the fungus from block to block was noted. In October, 1920, two of the tubes were opened and a representative block from each was sectioned and stained after the method outlined by Colley (1). Plate XXV, figure 2, shows the spore chain of *Torula ligniperda* distributed in the vessels, medullary rays and wood fibers of yellow poplar. Figure 5 plate XXV illustrates the manner in which the mycelium and spores follow the length of the cell. Microscopic examination of these stained sections showed clearly small hyphae passing through the cell walls, but as the safranin used in the stain colored both the tissue and the fungus more or less uniformly it was not possible to get a photograph which showed the penetration satisfactorily.

In addition to the tests described above a durability test was run for approximately six months, using essentially the method followed by Humphrey (4). At the end of the test, the blocks were split longitudinally and radial sections were made of the tissue in the middle of each block. Rapid microscopic examination of these sections was possible because the dark catenulate spores always contrasted strongly with the lighter wood tissue, and though no actual spore count was attempted, it was comparatively easy to determine the relative abundance of spores in the woods tested. The results of the examination are shown below.

Yellow poplar heartwood	Spores scattered sparingly in all blocks.
White ash heartwood	Spores distributed generally in all tissues of all blocks.
Cypress heartwood	Spores scattered sparingly in two out of four blocks.
Cucumber tree sapwood	Spores heavily distributed in all tissues of all blocks.
White ash sapwood	Spores heavily distributed in all tissues of all blocks.
Cypress sapwood	Spores scattered sparingly in all blocks.

The loss of weight in the infected blocks can only be stated in general terms. The test was evidently too short but, unfortunately it had to be discontinued. Sapwood lost more weight than heartwood by approximately 50 per cent in the yellow poplar and white ash blocks. In cypress the loss in heartwood and sapwood was about the same.

The fungus has been growing on a number of different kinds of nutrient agar for half a year and while sclerotial bodies have apparently developed there is not enough of this material to determine what these bodies are. It is a slow growing fungus. Optimum conditions for mycelium development appear to be 27° C. in darkness and, given these conditions, it has averaged 1 mm. a day for four weeks on 2.5 per cent malt extract agar.

Unfavorable physiological conditions, probably due to prolonged growth of the organism in the same culture or to a gradual drying up of the agar have repeatedly resulted in the production of spore groups of three and two, and even single spores; that is, under these unfavorable conditions the catenulate form disappeared. The size of the spore chain based on the measurement of ten mature spores is 10.5 to 12.7 by 45 to 72 microns. Figure 4, Pl. XXV shows a number of short spore chains taken from the margin of a Petri dish—a culture six months and fourteen days old. The hyaline spores are immature forms. In the figure, a number of simple unbranched conidiophores illustrate the common type of spore attachment. At "c" figure 4 Pl. XXV there is shown a slight constriction of the lateral hypha at the point of attachment. Another mycelium characteristic, a slight swelling of the hyphal joints, has been frequently observed, although it would appear that this is not a specific characteristic for the fungus, for Ellis and Bartholomew (2) describe subglobose joints in the hyphae of *Torula brachiata*. The comparatively sparse growth of mycelium in figure 4 Pl. XXV is characteristic of growth in attenuated cultures. Figure 1 Pl. XXV shows a number of four to six spore chains taken from the central part of the same culture as that from which the spores in figure 4 were taken, but which had developed at a period when the fungus was in full physiological vigor. The globose cell in the group in figure 3 is an abnormality which appears to be induced by retention of the fungus for a long time in the same culture. An interesting development in cypress test blocks of many short two and three spores chain suggest that cypress is a physiologically unfavorable medium.

Analysis of the results of the mechanical tests made on the yellow poplar and white ash described at the beginning of this paper, and careful comparison of the values obtained with sound and infected test pieces, showed that—

(a) In the case of the yellow poplar, it was practically impossible to correlate the differences in these results with the presence or absence of the fungus; but that

(b) in the case of the white ash all the infected logs except one "had a lower specific gravity than the average specific gravity of the sound logs; logs in which infection was found with difficulty showed little decrease in strength; and logs in which infection was readily found showed a marked decrease in strength values."<sup>1</sup>

Two points should be borne in mind in considering the above statements: first, that it was impossible to determine by means of culture tests whether or not any other fungus besides *Torula ligniperda* was present in the ash test pieces, because the ash had been kiln dried; and second, that *Torula ligniperda* has been reported by Willkomm, R. Hartig and von Schrenk (loc. cit.) as a concomitant of wood-destroying fungi, and observed in this laboratory associated with a brown cubical rot in eastern hemlock. *Torula ligniperda* was easily found in the ash by microscopical examination because the spores are typical. Hyphae of other fungi, had they been present, could not have been distinguished from the hyphae of *Torula* without culture tests, and it was impossible to make culture tests as pointed out above. Reduced to its simplest form the conclusion to be drawn from this discussion is that white ash, with plenty of evidence of the presence of fungous hyphae, but little or no evidence of breaking down of the wood cells, did show lower strength values than white ash in which no mycelium could be found.

#### SUMMARY

*Torula ligniperda* (Willk.) Sacc., a wood-inhabiting Hyphomycete, has been found in nature by various investigators in spruce, fir, oak, buckthorn, french briar, cypress, yellow poplar, maple, basswood, eastern hemlock, white ash and red gum. In the laboratory it has been grown in cypress, white ash, yellow poplar and cucumber tree.

The hyphae of the fungus, although usually running in the lumens of the wood cells, may occasionally penetrate the cell walls.

White ash logs heavily infected with *Torula ligniperda* showed a decrease in strength values as compared to values obtained from logs in which no infection could be found.

Culture studies have so far yielded no perfect form of the fungus.

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<sup>1</sup> Data supplied by Mr. R. F. Luxford of the Forest Products Laboratory.

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## DESCRIPTION OF PLATE XXV

- Fig. 1. Long four to six celled spore chains of *Torula ligniperda* produced near the center of a petri dish culture six and a half months old.  $\times 180$ .
- Fig. 2. Normal spore chains of *Torula ligniperda* distributed in the vessels, medullary rays and wood fibers of *Liriodendron tulipifera*.  $\times 120$ .
- Fig. 3. Globose cell in the spore chain—apparently a physiological reaction occurring when the fungus has been for a long time in the same culture.  $\times 525$ .
- Fig. 4. Short spore chains and hypertrophied mycelium obtained from the edge of a petri dish culture six and a half months old.  $\times 270$ .
- Fig. 5. Mycelium and three spore chains of *Torula ligniperda* following the length of the cells in *Liriodendron tulipifera*.  $\times 500$ .



*TORULA LIGNIPERDA* (WILLK.) SACC.



# LEAF SCORCH OR MOLLISIOSE OF THE STRAWBERRY<sup>1</sup>

R. E. STONE

WITH THREE FIGURES IN THE TEXT

The cultivated strawberry is subject to several leaf diseases. The best known of these diseases is the leaf spot due to the fungus *Mycosphaerella fragariae* (Schw.) Lindau. There are several other leaf diseases, as leaf blight, leaf blotch and leaf scorch. In the Province of Ontario a serious leaf disease is present in many fields. The disease has been named leaf scorch on account of the characteristic dry brown appearance of the leaves of the badly diseased plants (1). The name Mollisiose has also been applied (1). This disease has been observed in southern Ontario, the Niagara Peninsula and also in eastern Ontario. It has also been reported from several of the states in the United States.

## SYMPTOMS OF THE DISEASE

In the early part of the season, May, the strawberry leaves show irregular purple blotches ranging in size from  $\frac{1}{8}$  to  $\frac{1}{4}$  inch in diameter. There may also be purple stripes on the petioles and on flower peduncles. In this stage the disease resembles the early stage of "leaf spot" but the blotches are much more irregular. As the season advances the blotches enlarge and coalesce. In time the whole leaf may be involved. As the blotches increase in size they become grey or cinereous, with a purple border. Later the definite border disappears. When the blotches have become dry and cinereous the dark acervuli become prominent, scattered thickly over the surface. As the disease progresses all the leaves on a plant take on a dry burned appearance (Fig. 1). It is not uncommon in July and August to find strawberry beds which have the appearance of having been burned over. Leaf scorch is seldom very prominent in new plantings except on a few plants, as any plant showing pronounced indications of the disease is usually discarded. However, where susceptible varieties are being used most of the plants will show some blotches before the end of the season. The next spring the disease appears very early and may be quite serious before the crop is picked. After the harvest the disease progresses very rapidly and all the leaves may be dry and cinereous by July or August. The diseased plants do not winter well and the crop may be very short the second year.

<sup>1</sup> Contribution from Botanical Department, Ontario Agricultural College, Guelph, Ontario.



## VARIETAL SUSCEPTIBILITY

Not all varieties are equally susceptible to the disease. In fields where several varieties are being grown the difference in the susceptibility of the different varieties is very marked. One variety may be badly affected while another may show scarcely any disease. It is not unusual for a grower to have two or more varieties in his plantings so that several varieties may be directly compared. From a study of plants in the field the common varieties may be arranged in the following order.

Very susceptible	Moderately susceptible	Slightly susceptible
Clyde	Senator Dunlop	New Williams
Glen Mary	Ruby	Portia
Doctor Burrill	William Belt	Parson's Beauty
Pokomoke		Enhance
		Vanoise
		Joe

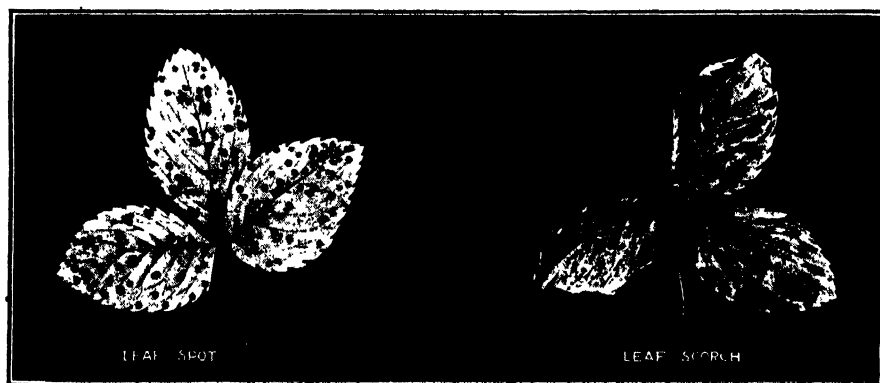


FIG. 1. A drawing showing the difference in the appearance between "leaf spot" and "leaf scorch."

## CAUSAL ORGANISM

An examination of the cinereous portions of leaf blotches and diseased petioles reveals the presence of dark acervuli which are filled with hyaline two-celled spores. The fungus agrees with the description of *Marsonia potentillae* (Desm.) Fischer, and also agrees with exsiccated specimens of this fungus.<sup>1</sup> The fungus can be grown readily in culture on the ordinary media. When strawberry leaves are inoculated from a pure culture they develop the typical leaf scorch.

<sup>1</sup> Examined by Miss Anna E. Jenkins, U. S. Dept. Agr. B. P. I.

## OVER-WINTERING THE FUNGUS

When strawberry plants are mulched many of the leaves remain green over the winter. In these leaves the fungus winters over in the vegetative condition and produces abundant conidia in the spring. On some of the dry leaves the conidia remain in the acervuli. These conidia retain their vitality until the following spring. They germinate, in hanging drops, in about 6 hours, producing germ tubes from one or both cells of the spore.

On the dry leaves there is also produced an ascigerous stage. This stage occurs in abundance on the more exposed leaves. The earliest ascocarpus mature in late April and may be found until June. The perfect stage agrees well with descriptions and exsiccati specimens of *Mollisia earliana* (E. & E.) Sacc.

## EVIDENCE OF THE RELATION OF MARSONIA POTENTILLAE AND MOLLISIA EARLIANA

*Mollisia earliana* is found on dry strawberry leaves following *Marsonia potentillae* and the conidia are often present on the leaves at the same time as the ascocarps.

## RESULTS OF CULTURES FROM SINGLE ASCOSPORES

Cultures of the Marsonia stage were secured from single ascospores.

Date	No. of ascospores spores	No. of colonies	Spore form	Date
May 15, '19	15	10	Marsonia	May 29, '19
May 21, '19	10	7	Marsonia	June 4, '19
May 7, '20	15	14	Marsonia	May 21, '20

In all cases where the ascospores germinated a pure culture was obtained which produced spores of the Marsonia type. Strawberry plants were inoculated with a spore suspension obtained from some of these colonies. June 10, 1919, ten strawberry plants were inoculated by spraying with a spore suspension taken from the colonies obtained from a single ascospore planting made May 15, 1919. June 15, purplish spots were plainly seen. June 17, the spots were prominent, the centers becoming cinereous. June 21, the spots were typical of Marsonia. June 24 acervuli, bearing typical spores of *Marsonia potentillae* were present. Control plants remained free from disease.

May 26, 1920, five plants were inoculated with a spore suspension taken from the culture obtained from an ascospore planting made May 7. May 29, spots were evident. June 4, the spots were well

developed. June 9, fruiting, acervuli and spores typical of *Marsonia potentillae*. Control plants remained free.

June 16, 1920, ten plants were inoculated with a spore suspension taken from the same culture as that used on May 26. June 21, the spots were evident; June 26, spots extensive. July 1, typical acervuli and conidia of *Marsonia*. Control plants remained free from disease.

As the fungus causing leaf scorch or Mollisiose has not been adequately described the following description is appended.

(4) *Mollisia earliana* (E. & E.) Sacc. Syll. Fung. 8: 328

*Peziza earliana* E. & E. Bull. Tor. Bot. Club 1884, p. 74

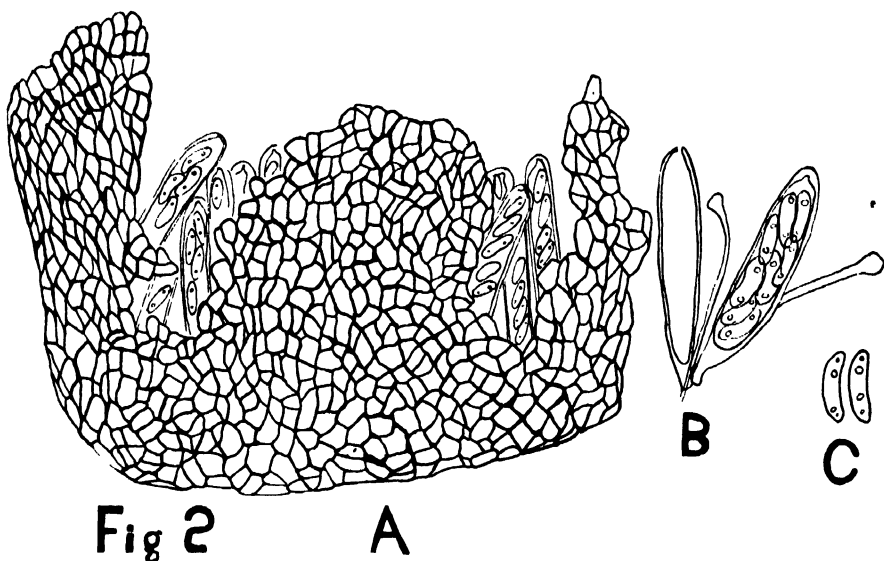


FIG. 2. A. Perithecium of *Mollisia earliana* (E. & E.) Sacc. showing the manner of dehiscence. B. Asci and paraphyses, one ascus empty showing the pore, the other containing spores and showing the slightly thickened apex. C. Typical ascospores.

(3) *Phyllosticta potentillae* Desm. Am. Sc. Nat. 8: 31

*Leptothyrium dryadearm* Desm.

*Septoria potentillarum* Fück.

*Gloeosporium potentillae* (Desm.) Oud. 26 Jarg. Ned 3, Beil p. 3

*Marsonia potentillae* (Desm.) Fisch. Rabenh F. Eur. 1857

(5) *Marsonia fragariae* Sacc. Malpighia 1896: 276.

(6) *Ascochyta fragariae* Auct.

*Ascochyta colorata* Auct.

#### ASCIGEROUS OR PERFECT STAGE

Apothecia hypophyllous superficial, brown to black, membranaceous, 0.25–1 mm. in diameter splitting open irregularly, margin irregular,

closing when dry and resembling a perithecium. Hymenium pinkish yellow. Asci 55–70  $\times$  14–25  $\mu$  fasciculate, cylindrical, wall slightly thickened at the apex, opening by a pore. Ascospores hyaline, one-celled, 2–4 guttulate, elongated-elliptical, slightly curved, closely packed in the ascus, 18–28  $\times$  3–6  $\mu$ . Paraphyses slender, capitate, non-septate, simple 50–60  $\times$  3–3.5  $\mu$ ; head 4–5  $\mu$  broad. (Fig. 2.)

On dry, overwintered leaves of cultivated strawberry, following *Marsonia potentillae* (Desm.) Fisch.

Collections—Guelph, Ont., May 14, 20, 22, 1920.

Guelph, Ont., Apr. 28, May 3, 8, 16, 26, 1920.

Original material collected by Earle in Illinois on *Fragaria*.

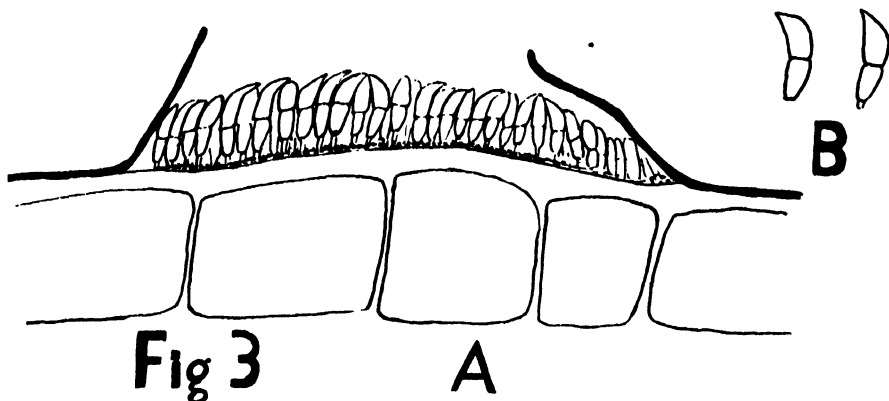


FIG. 3. A. Acervulus of *Mollisia earliana* (E. & E.) Sacc. (*Marsonia potentillae*) with conidia showing the sub-cuticular character. B. Typical conidia.

#### CONIDIAL STAGE

Spots at first purplish, irregular, later becoming cinereous in the center with a purplish margin, 4 to 12 mm. in diameter, becoming confluent and often involving entire leaves, also appearing as purplish streaks on petioles and peduncles. Badly diseased plants take on a dry scorched appearance.

Acervuli sub-cuticular, dark when dry, scattered over the cinereous portions of spots, glistening when moist; spore mass gelatinous, glistening, pink.

Conidia hyaline, two-celled, asymmetrical, upper cell larger, ros-trate, 24–30  $\times$  5–8  $\mu$ , (Fig. 3).

Collections—Guelph, Ont., May 20, June 12, Sept. 8, and 29, 1919.

Guelph, Ont., Apr. 28, May 3, June 18, July 20, 1920.

London, Ont., Aug. 14, 1920. Waterford, Aug. 16, 1920.

Brantford, Aug. 18, 1920. Peterborough, Aug. 24, 1920.  
Ottawa, Aug. 28, 1920.

Guelph, Ont. May 1921, Burlington and Bronte and Port  
Nelson, June 13, 1921. Grimsby, St. Catharines,  
Jordan Harbor, June 16, 1921.

Also reported from Louisiana (Edgerton) Delaware (Cook), New  
Jersey (Cook) Connecticut (Clinton) Indiana (Jackson) (6) Illinois,  
(Anderson)<sup>1</sup> Europe (3), Siberia (5).

Control measures have not been worked out for this disease but it is  
reasonable to suppose that it can be checked by using the same measures  
used to control the leaf spot.

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1921.

<sup>1</sup> Specimen received.

# THE LIFE HISTORY OF ROSELINIA CARYAE SP. NOV. CAUSING A HICKORY CANKER AND DISEASE

LEE BONAR

WITH THREE FIGURES IN THE TEXT

Early in the spring of 1921 Dr. C. H. Kauffman collected in some woods near Ann Arbor, Michigan, some twigs of *Carya ovata* which showed dead sunken areas on what were otherwise vigorously growing young shoots. An examination of this material by the writer showed these dead areas to contain the fruit bodies of a fungus. A survey of this wood lot, in which there are numerous saplings and young trees of

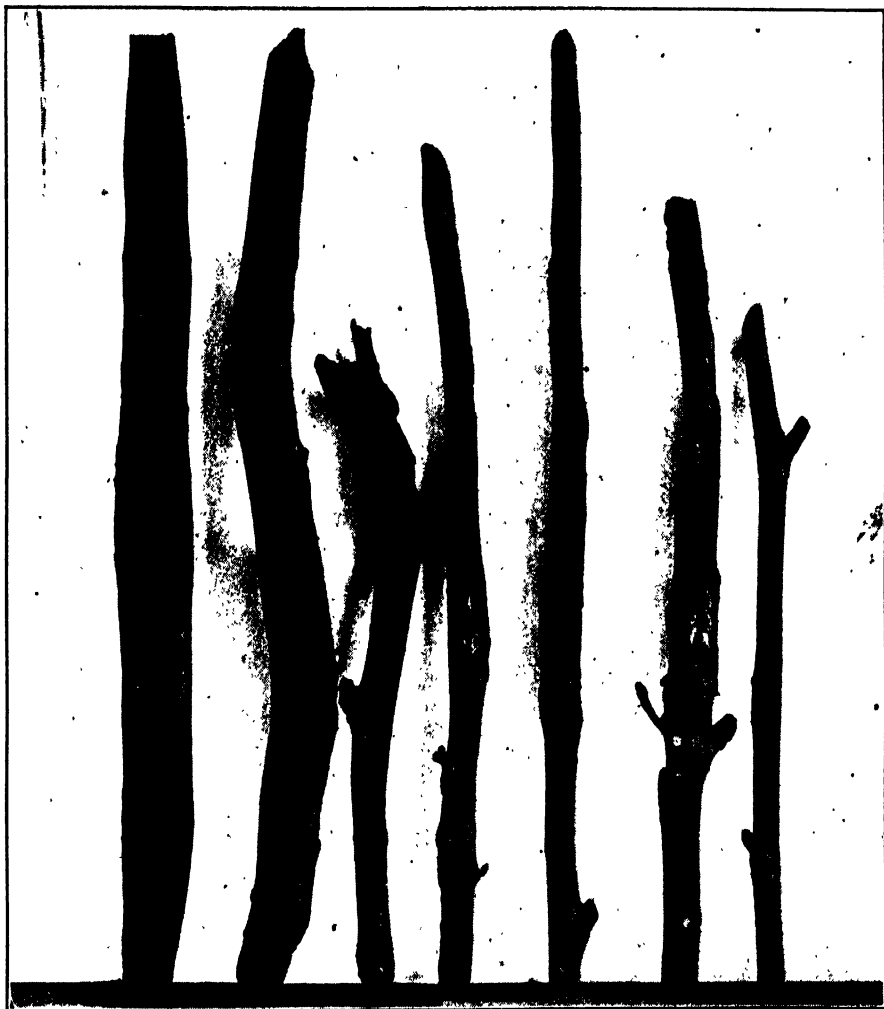


Fig. 1. Photograph of twigs showing cankers in varying stages of development.

*Carya ovata* showed a high percentage of the younger growth of this species, up to 4 inches in diameter, stump high, to be cankered to a greater or less degree.

The cankers on the young twigs are almost always around the leaf scars, or on the tips. (Fig. 1.) They are often quite distinct around each leaf scar, while in other cases they are confluent and the whole twig may be dead especially near the growing tip, where the killing is more extensive. As a rule the cankers are sunken areas, where the bark is still intact, and there is a raised margin in the formation of the healing lines about the border of the canker. The cankers are of varying sizes, from mere spots less than an inch in length, up to as much as 3 x 6 inches on some of the larger trunks. The cankers are, in practically all cases of greater extent up and down the twig than in a lateral direction, and except on very young twigs, seldom girdle the twig. Infection in small lateral twigs often extends to the main branch where it forms a dead sunken area about the point of union, but does not extend into the larger branch very far. The cankers on the larger limbs and on the trunks of these young trees are various in form. They were not observed on trunks more than 4 inches in diameter, stump high. As a rule the heavy bark is persistent over these sunken dead areas on the trunks, and when rather numerous they give the tree a gnarled and distorted appearance. One of these young trees presents, as a rule, a scraggly, irregular top, due to the fact that the leader has been killed, and sometimes the first lateral ones also. This combined with a gnarled trunk, in extreme cases, gives a very scrubby young tree. A few cases were seen where very much gnarled saplings 2 to 3 inches in diameter had died back to the ground. In such cases a whorl of sprouts had come up about the dead trunk, and these showed numerous small infections on the tips and at the leaf scars. Some of these sprouts 5 years old produced new leaders and presented the typical scrubby appearance.

#### THE FUNGUS AND THE HOST

Examination of the cankers on the younger twigs invariably showed abundant fruiting bodies of a fungus in the bark. They are formed just beneath the periderm, and break through singly as small pustules. No fruit bodies of the fungus were found in the cankers on the trunks, in the very hard heavy bark. These cankers on the trunks were in all cases, however, associated with an abundance of twig infection with the fruit bodies of the fungus present, so that there seems no doubt that they are of the same origin. These fungous fruit bodies prove to be pycnidial structures, somewhat variable in form, growing just beneath

the periderm which then breaks and shows a black dome beneath. In section one sees a black differentiated wall over the top only (Fig. 2e). Subsequent growth, however, tends to form a more definite pycnidial structure as the black wall develops downward all the way around except a narrow portion at the base. (Fig. 2f.)

There is no definite ostiole, but the hymenial layer extends over the entire inner surface of the pycnidium. Hyaline spores are borne in great abundance on the short conidiophores which are shaped like Indian clubs, (Fig. 2g) and are continuous around the entire interior of the fruit body. These spores are broadly fusoid, usually containing oil globules, and are  $5$  to  $7 \times 2.5$  to  $3 \mu$  in size. At maturity the pycnidium bursts open in an irregular manner. Sometimes the spores emerge through an irregular split, more often, however, the whole upper carbonaceous part breaks away as a lid leaving a cuplike structure.



Fig. 2. e-young pycnidium, f-mature pycnidium, g-conidiophores and spores, h-pycnidiospores.

Single spore isolations were made from this fungus, and it was found to grow readily in culture. It fruits abundantly on sterilized green bean pods, or green bean agar, but not on corn meal or sugar-peptone agar. Pycnidia formed in artificial culture are found to have a pycnidial wall all the way around, and in some cases there is a slight papilla like growth similar to an ostiole, although no opening has ever been observed. Clean hickory twigs in test tubes, were sterilized and inoculated with a pure culture of the fungus. They became covered with an abundant growth of mycelium and pycnidia formed in the mycelial growth.

#### THE ASCUS STAGE

Young twigs, normally infected, were brought in from the woods, and sterilized on the outside by soaking in a 1-1000 solution of mercuric



chloride for 5 minutes. After this they were washed very carefully in large test tubes containing sterile water, through two washings, and then placed in sterile tubes containing a small amount of sterile water. After 6 months numerous fruit bodies were found developing on the bark of one of these twigs. Examination showed these to be perithecia bearing mature ascospores in abundance (Fig. 3, a-c.) Single spore isolations were made from these ascospores, and the resulting cultures gave a pycnidia forming fungus corresponding morphologically and culturally with the fungus isolated from the cankers of the twigs.

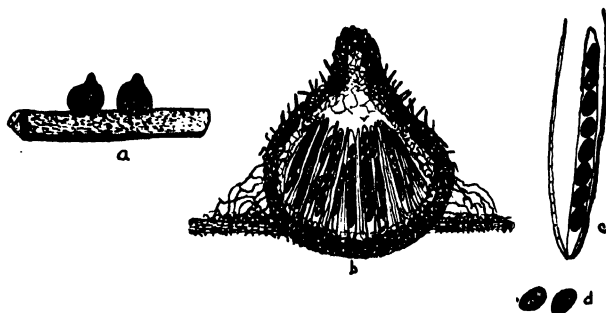


Fig. 3. a-habit of the perthecium on the wood, b-median section through perithecium, c-ascus and paraphyses, d-ascospores.

#### IDENTITY OF THE FUNGUS

The ascus stage of this fungus falls into the genus *Rosellinia*. As this species appears to be undescribed in the literature, the name *Rosellinia caryae* sp. nov. is proposed for it. The taxonomic position of the imperfect stage of this fungus is not so easily determined because of the variance of the opinion of different writers on the general group to which it belongs. It falls in the genus *Dothichiza* Sacc. (4), in the sense of Diedecke (1). Diedecke, in his treatment of the subject, thinks that this genus should be placed in the Sphaeropsidales, but hesitates to do so because of the dearth of information concerning many species of the genus *Dothichiza* (3). The form in hand comes very close to the genus *Phomopsis* in the sense of Diedecke and Von Höhnelt, as Diedecke (2) says is true of all the known species of the genus *Dothichiza*. The principal argument against connecting this form with the genus *Phomopsis* is that, in all the known cases, the ascus stage of the species of *Phomopsis* belongs to the genus *Diaporthe*.

#### TECHNICAL DESCRIPTION

***Rosellinia caryae* sp. nov.** Perithecia superficial or slightly sunken in the outer bark, scattered or gregarious, carbonaceous, brittle, slightly

bristly in some cases, otherwise smooth, broadly flask-shaped, with a short distinct ostiole. Asci cylindric, 8-spored, averaging  $50 \times 6-8 \mu$ . Spores uniseriate, sub-globose, one-celled dark brown,  $5-7 \times 3.5-4.5 \mu$ . Paraphyses tenuous filiform.

#### IMPERFECT STAGE

**Dothichiza caryae** sp. nov. Pycnidia scattered, erumpent, dome-shaped, non-ostiolate, irregularly dehiscent, wall distinct except at the base, hymenial layer continuous over the inner surface, conidio-phores short, Indian club shaped, spores 1-celled, hyaline, broadly fusoid,  $5-7 \times 2.5-3 \mu$ .

#### SUMMARY

1. An undescribed canker and disease is found on young trees of *Carya ovata*.

2. Cankered areas are most abundant on the smaller limbs and twigs, but are sometimes found on the trunks of saplings.

3. A pycnidium forming fungus is constantly associated with the cankers on the twigs.

4. The ascus stage of this fungus was developed on hickory twigs in the laboratory, and cultures made from single ascospores correspond identically with cultures made from the pycnidial forms on the twigs.

5. The name *Rosellinia caryae* sp. nov. is proposed for this fungus, the pycnidial stage of which belongs to the genus *Dothichiza*.

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## LIGHTNING INJURY TO HEVEA BRASILIENSIS

CARL D. LA RUE

Rutgers<sup>1</sup> has compared the effect of lightning on Hevea trees with that caused by the same agent on various trees in Europe and has discussed in some detail four types of injury. These four types may be summarized as follows:

1. In this case single trees or groups of trees are killed, while the surrounding trees may show injury resembling the so-called "die-back."

2. This type of injury manifests itself as a rather long, vertical split in the bark.

3. A number of small wounds characterize this type of damage. These wounds resemble those caused by severe hail storms. They are usually in the upper part of the tree and when the exuded latex turns dark they become very conspicuous.

4. This type of injury consists in the killing of the outer layers of the bark which then slough off giving the tree a scurfy appearance.

The writer has little to add to Rutgers' description of the last three types of injury but certain phases of the first type have come under his observation which have not yet been described.

Rutgers reports that only on one occasion has he seen young trees killed by lightning. The writer, however, has seen a number of cases of this sort in trees from one to three years old. Attention is usually attracted to the injured trees by the wilting of the leaves about a foot from the tip of the stem while those above and below this point are still normal. Examination shows that the bark of the twigs bearing the withered leaves is dead and black. The cambium of the regions immediately above and below the dead bark is discolored, generally showing a purple hue, while the cortex still appears green and normal. The discoloration of the cambium progresses upward to the tip of the stem and downward to the root and in a few days the tree is dead.

By the time this stage is reached, (frequently before the injury is noticed) the part of the bark which first showed injury is covered with *Diplodia* spores and often the case is diagnosed as "*Diplodia* die-back."

Frequently a number of trees are killed in one spot, although sometimes only one, and a number of neighboring trees are injured only to the extent of losing a foot or two of the tips of their stems by "die-back."

<sup>1</sup> Rutgers, A. A. L. Bliksemschade bij Hevea. Archief voor de Rubbercultuur in Nederlandsch-Indie. 3: 163-170. 17 fig. 1919.

If seen in its early stages the injury can be easily diagnosed by the purple discoloration of the cambium, and by the curious fact that the tip of the stem almost always (always in the writer's experience) appears entirely normal while the bark just below is dead. Perhaps the denser rosette of leaves at the tip in some way protects the stem at that point.

The same type of injury in mature trees had become familiar to the author before it was observed in young plantations. In the first case lightning was not at first suspected as being the cause. The tree most affected had one branch in its top which was dead with all its leaves withered. All of the bark of this branch was dead, and from the point where the branch joined the trunk to within 3 feet of the root crown, all the bark of the trunk was dead.

The limits of the diseased bark were determined as accurately as possible. Beyond the totally dead bark an area of sound bark was found which overlaid discolored cambium. The cambium under the dead bark was black; that underlying the apparently sound bark was of a deep purple color which became fainter and fainter until it finally disappeared entirely as the limit of the injured area was reached.

The adjacent trees were examined and it was found that most of the branches nearest the dead branch of the first tree were affected. A few leaves just back of the tip of one twig of each of these branches were wilted and dead. The leaves at the tip were usually normal or just beginning to wilt. The bark of the region bearing the wilted leaves was found to be black and dead while beyond this region the cambium showed the purple discoloration for a distance of about 2 feet. The remainder of each of these trees was entirely normal. It looked very much as though an infection was spreading from the diseased branch on the first tree to all the surrounding trees.

Two days later the trees were again examined. All the bark which showed injury at the time of the first examination was now dead and in many places it was covered with *Diplodia* spores. The cambium under the dead bark was full of black mycelium resembling that of *Diplodia* and the vessels of the wood beneath for a depth of 1 or 2 mm. were filled with mycelium making them appear as black lines.

In the tree first observed the damage had extended throughout the bark of all of the branches, of the trunk, and of the main roots. The cambium layer showed the purple color already described and had a strong, sour smell much like that caused by *Sphaerostilbe* infection.

In the less severely affected trees it was found that the discoloration of the cambium had progressed downward from the twigs; in some cases for only 3 or 4 feet, in other cases to the ground level.

Bits of tissue from the diseased areas, and of sound tissue bounding the diseased areas, were allowed to develop in agar tubes. These cultures included cambium, wood and bark, from twigs, trunks, and roots. Almost without exception these cultures developed growths of *Diplodia cacaoicola*, with which in a few cases *Gloeosporium alborubrum* and other common parasites were associated.

However, knowing that almost any tissue, cut from apparently healthy *Hevea* trees, will develop *Diplodia* mycelium and spores as it dies, the author was unwilling to accept the evidence of the cultures and to believe that *Diplodia* was able to produce such rapid and destructive effects. A few days later the wire cup hanger on the trunk of a tree showing similar injury was found to have been melted by lightning, and the clue given by this was followed until conclusive evidence was secured.

It is very likely that it was the absence of any indication of the action of lightning, together with the development of *Diplodia* on injured trees in the field, and in cultures of diseased tissues which led Petch<sup>1</sup> to suspect that *Diplodia* was the cause of the death of such trees. He believed that the organism concerned was an extremely virulent species of *Diplodia*, which he named *Diplodia rapax*. From the evidence given above there can be little doubt that the trees described by Petch were killed by lightning.

The spread of the discoloration in the cambium and bark is curious and closely resembles the progress of an infection. It appears that the path of the lightning is not on the surface of the tree, as it usually appears to be in injuries to trees in Europe and America, but through the cambium. This tissue seems to offer the best path for the conduction of electricity. However, it may be that the current passes mainly through the water in the vessels of the sap-wood and that the wood does not readily show the injury. The cambium which lies nearer the sap-wood than does the phloem or the cortex shows evidence of derangement earlier than either.

The region which carries the heavier part of the current is likely killed at once; the cells which carry a weaker current die more slowly and this gives an apparent effect of a spreading infection. Since a branch has a smaller cross section area for the current it carries than has the trunk of the tree, the branch dies near the tip first and the death of the tissues progresses downward to the roots. In many cases the current is ap-

<sup>1</sup> Petch, T. The physiology and diseases of *Hevea brasiliensis*, Dulau & Co. Ltd. London, p. 1-268. Pl. 1-15. 1911.

parently only strong enough to kill the twigs or smaller branches, while the trunks and larger branches are not noticeably injured.

At the time the trees described were found some 30 trees were seen within an area of about one-tenth of a square mile which were all either killed or severely injured by the same electric storm. These injured trees were in groups of 5 or 6. Similar groups of damaged trees were reported in several other areas in the vicinity. The exact nature of the discharges causing the damage is not known but it is probably not the noisy type of discharge common in America, as thunder storms are very rare in Sumatra. It is more likely some form of silent discharge, though silent discharges are usually considered harmless.

The purple color developed in the cambium which has been killed by lightning has already been sufficiently emphasized. This is of value in diagnosing lightning injury in *Hevea* trees as it is very rarely developed in cambium killed by other agents. This color often extends outward into the bark nearly to the cork.

After the bark is dead it is markedly different from bark killed in other ways. The bark of *Hevea* is always full of stone cells, and in bark killed by lightning all of the other cells disintegrate within a remarkably short time leaving nothing but the stone cells with the strands of rubber which have coagulated in the latex vessels. The nature of the bark is a sufficient indication of lightning injury in cases where it is too late to detect the characteristic discoloration.

Without doubt a great many cases of "dic-back" in the tops of *Hevea* trees are due to lightning but are erroneously attributed to *Diplodia* or some other organism.

Rutgers states that he has never seen branches or strips of wood and bark torn from *Hevea* trees by lightning. The writer has observed three cases of this type of injury but it is extremely rare.

#### SUMMARY

Trees of *Hevea brasiliensis* are frequently injured or killed by lightning. Such cases of injury are usually wrongly diagnosed. Both young and old trees may be affected. In some cases a differential rate of death of the injured tissues closely simulates the effects from the invasion of the tissues by a destructive organism. Splintering or shattering of trunks or branches by lightning is extremely rare in rubber trees.

# THE EFFECT OF LACTIC ACID ON SPORE PRODUCTION BY COLLETOTRICHUM LINDEMUTHIANUM<sup>1</sup>

E. F. HOPKINS

WITH TWO FIGURES IN THE TEXT

It has been the experience of the writer in using *Colletotrichum lindemuthianum* (Sacc. & Magn.) Bri. and Cav. in class work that the organism does not sporulate readily on potato dextrose agar. Barrus (1, 2) states that it sporulates readily on bean plugs or in culture media at 21° C. when the cultures are "in good spore producing condition," but in carrying a culture of the B strain of this species for over a year on potato agar in this laboratory with frequent transfers no macroscopic pustules of conidia were formed, although the same culture fruited abundantly on bean plugs. At the same time cultures of two other anthracnose organisms, *Colletotrichum trifolii* Bain, and *Gleosporium musarum* C. & M. fruited abundantly on this medium. In the case of *Colletotrichum pisi* Pat., Jones and Vaughan state that this species does not fruit readily on common media.

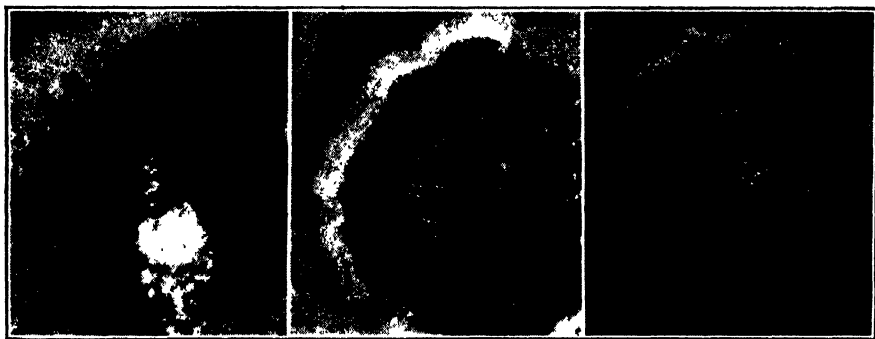


FIG. 1. Lactic acid and spore production in *Colletotrichum lindemuthianum*: 1, 2, and 3 drops of lactic acid respectively per 20 cc. of potato agar.

A preliminary experiment was carried out to determine the effect of lactic acid on spore production in this species. Transfers were made to ordinary potato agar and to potato agar containing 1, 2, and 3 drops of 50 per cent lactic acid (approximately 7 N) per 20 cc. of the medium. On the eighth day after planting the cultures, abundant sporulation could be observed macroscopically in the culture to which 3 drops of acid were added. This showed as a pink slimy layer about 1 cm. in diameter while the colony was about 1.7 cm. in diameter. In the cul-

<sup>1</sup>Published by permission of the Director of the Missouri Experiment Station.

ture containing 2 drops of acid a few pustules were observed while in the one with one drop no sporulation was visible to the naked eye although some conidia were found by making a microscopic examination. The appearance of the fungus on agar to which no acid had been added was similar to the last.

In figure 1 the cultures containing 1, 2, and 3 drops of acid are shown. These were photographed several days after the above notes were taken. By this time the culture with 2 drops of acid had sporulated to a somewhat greater extent than before, but the one with one drop showed no macroscopic evidence of sporulation even after being kept for some time. The whole surface of the culture to which no acid had been added was scraped off and mounted in liquid for microscopic examination. Only a few spores were found in the entire mount. The spores formed in the most acid plate were normal in shape and size.

After this same procedure was used successfully by students in obtaining abundant fruiting in petri dish cultures, the experiment was repeated. At the end of 8 days abundant sporulation was noted in the most acid culture and none in the others. At the end of 10 days there were a few pustules in the culture containing two drops of acid. In order to estimate the number of spores produced in these cultures after 10 days of growth the diameters of the colonies were recorded and direct counts of the spores were made. To accomplish this suspensions of the spores were made from each plate by pouring a few cubic centimeters of a weak solution of para cresol over the colony and the spores loosened by gently rubbing the surface of the culture with a scalpel. The spore suspension thus formed was poured through a funnel into a graduated cylinder and the surface of the plate washed carefully with more of the cresol solution until the volume amounted to 20 cc. The purpose of the cresol was to prevent germination and contamination while making the counts. The spore suspensions showed increasing turbidity from the least acid, which was practically clear, to the most acid culture which was very turbid.

After shaking the spore suspensions thoroughly counts were made in a haemocytometer. It was found advisable to count all the spores found in the nine square millimeter area of each cell except in the case of the most acid culture in which case 8 of the smaller squares in the cell were counted and averaged and the total number for the counting chamber calculated. The volume of each of the two cells was 1.68 cu. mm. From the counts made in this manner the total number of spores in the 20 cc. of spore suspension was estimated and by dividing by the area of the colony in square centimeters the results were reduced to



comparative basis. The data are summarized in table 1. The pH values given were not determined but are approximate and are taken from a titration curve for potato dextrose agar recently presented by the writer (4).

TABLE 1

*Effect of lactic acid on spore production by Colletotrichum lindemuthianum*

Drops of 50 per cent lactic acid	pH approximate	Diameter of colonies in mm.	Spore Count			Total number of conidia	Conidia per sq. cm. of colony
			I	II	Average		
0	7.4	33	0	6	3	35,714	4,176
1	4.5	40	174	181	177.5	2,113,100	168,200
2	4.0	37	244	265	254.5	3,029,800	281,800
3	3.8	28	4262	3960	4111	48,941,000	7,947,000

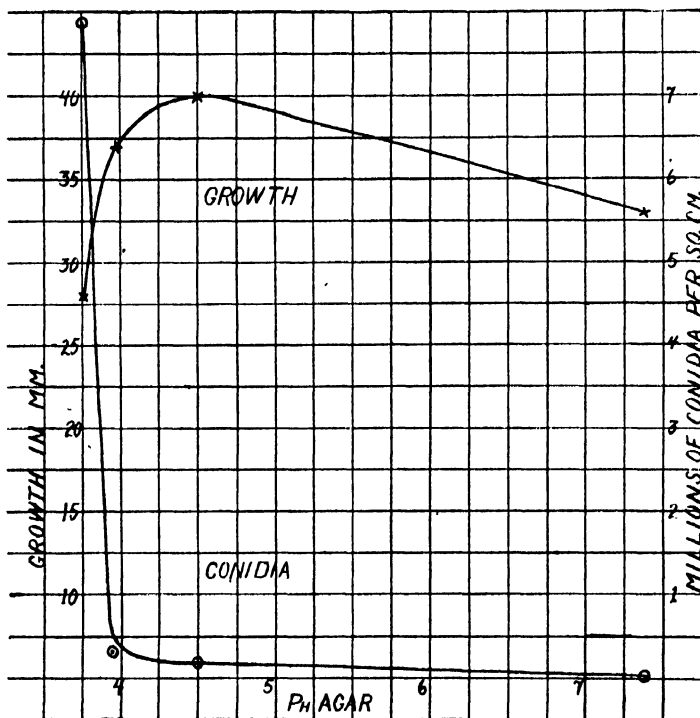


FIG. 2. Lactic acid and spore production in *Colletotrichum lindemuthianum*: graphical representation of growth and of conidia per sq. cm. of colony surface.

The errors are multiplied to a large extent but the comparative difference is easily seen. This is brought out more clearly in figure 2. Spore production in this instance increases gradually with increasing initial hydrogen-ion concentration from about pH 7.4 to about 4.0 and then takes a sudden jump at 3.8. It is noteworthy that with this great increase in spore production the increased acidity does not greatly inhibit the amount of growth. The growth in diameter in the most acid culture is 0.7 of that of the largest colony in the series. Spore production in this species therefore, appears to increase with increase in hydrogen-ion concentration and with the accompanying decrease in the amount of growth.

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## ON THE OCCURRENCE OF MYCOSPHAERELLA WILT OF MUSKMELONS IN JAPAN

TAKEWO HEMMI

The wilt caused by *Mycosphaerella citrullina* (Smith) Gross. is a well-known disease which is occasionally destructive to greenhouse muskmelons and to watermelons in America. Until 1919 the writer had never heard of the outbreak of this disease in Japan. August 19, 1919, Dr. Hoshino, Professor of Horticulture of Kokkaido Imperial University at Sapporo, called the attention of the writer to the outbreak of an uncommon disease which was causing injury to the muskmelons being grown in the greenhouse of the Department of Horticulture (3). The writer visited the greenhouse and found several plants attacked by a disease which was quite new to him. The diseased plants were as green as the healthy plants in general appearance, but several of their lower nodes were pale-green in color with or without gum exudation. Some of the diseased areas had reached a more advanced stage, being dry and blackish or grayish in color. On such dried areas the writer found abundant minute black points which proved to be the pycnidia or the perithecia of a kind of fungus. In this case the cross-section of the vine at the diseased region showed discoloration only at the outer portion of the primary cortex and therefore indicated the disease to be in the early stage of its development. Subsequently the writer visited the greenhouse several times and learned that the disease had spread very rapidly, causing the wilt of leaves and vines, and occasionally becoming so severe as to result in the complete ruin of the plant. At that time the infected areas could be seen also at the upper portion of the vine, but most of these infections, having originated in the crotch of a branch or in the axil of the leaf, were confined to the vicinity of the nodes. Although the writer was out of the city in the summer of 1920, he was informed that the disease had reappeared in the same greenhouse and had also broken out in the greenhouse of the botanical garden belonging to the same university.

The symptoms of the disease are shown first as pale-green and irregular-shaped patches around the nodes. The diseased areas rapidly extend some distance above and below these infected nodes and soon a reddish brown gum exudes from several points on these areas. The areas gradually become gray or blackish gray and the exuded gum dries and sometimes turns blackish-brown. Owing to the abundant development of

the pycnidia and also of the perithecia of the causal fungus, the diseased vine in the advanced stage often appears black. The diseased regions extend sometimes to the leafblades through the petioles, showing discoloration and minute black points on the upper surface and also on the petioles. The diseased areas on the leaf are generally large and irregular in shape, light brown or grayish brown in color, and indistinct in demarkation. The diseased leaves gradually shrink, turn yellow, and finally shrivel up. The careful observations of the symptoms as well as the microscopical studies of the fungus developed on the vines led the writer to recognize the disease to be the same as the so-called "Mycosphaerella Wilt" described by Grossenbacher (2) in 1909.

In the fall of 1890 the watermelon vines cultivated on a field in the eastern United States were attacked by a severe disease and the causal fungus was recognized by Chester to be a species belonging to the genus *Phyllosticta*. He examined a similar disease in 1892 and found again the same fungus, and also an ascigerous fungus side by side with it in the same spots which he thought was a species of *Sphaerella* (1). In 1905 Smith (7) reported that during the autumn of 1903 he had found the same fungus most abundantly on the leaves and the fruits of squash and pumpkin and also to a limited extent on the leaves of cucumber and cantaloupe. He changed the genus name of this fungus and called it *Ascochyta citrullina* (Chester) Smith in its pycnidial stage, using the name *Sphaerella citrullina* Smith for the perfect stage. According to Grossenbacher the fungus on several of the cucurbits reported by Chester and Smith is nothing but the causal fungus of the wilt of greenhouse muskmelon under consideration. It was in 1907 and 1908 that the fungus attacked severely the muskmelons grown in one of the greenhouses of the New York Agricultural Experiment Station, where Grossenbacher found opportunities to study the disease. In the early summer of 1909 Massee (4, 5) in England several times received the diseased stems of tomato, on which he found also the same fungus. So far as the writer knows, this is the only report of the fungus attacking other plant besides cucurbits. Soon after that some cucumber vines attacked by the same fungus, were received. By inoculation experiments it was proved that the fungus on cucumbers affects tomato seedlings and that the fungus on tomatoes affects pumpkin seedlings. According to Grossenbacher, however, cucumbers are immune to the disease; consequently the writer was interested to investigate further the pathogenicity of the fungus to cucumbers. At the last meeting of the American Phytopathological Society at Toronto in December 1921 Meier, Drechsler and Eddy (6) presented a paper on the cucumber black rot caused by

the fungus. They found the disease on cucumbers shipped from Florida on their arrival at the New York market. Although the pathogenicity of the fungus to cucumber vines is still unknown, their discovery of the fungus on the fruit is important.<sup>1</sup>

By microscopical examinations the writer recognized the morphological characters of the fungus found in Japan to be quite identical with those described by Grossenbacher and several other authors. The pycnidia and also the perithecia develop first in the tissue and elevate the epidermis, but soon they perforate it and appear on the surface. The pycnidia measure about 82 to 164  $\mu$  in diameter. The pycnospores are hyaline, mostly two-celled, 7 to 20 by 3 to 6  $\mu$  in size and more or less constricted at the septa. The cells in these two-celled spores are some times unequal in size. The spores seem to vary considerably in size and also in shape. Mixed with the predominating two-celled spores the writer also found one-celled, three-celled, or four-celled spores. The shape of the spores is generally more or less cylindrical, ovoid, or clavate, mostly with rounded and sometimes with tapering ends. This pycnidial stage is called *Ascochyta citullina* (Chester) Smith or *Diplodina citrullina* (Smith) Gross. The perithecia measure about 80 to 160  $\mu$  in diameter, and in their vigorous condition often become almost wholly superficial. The asci are 44 to 56 by 7 to 11  $\mu$ , cylindrical or clavate-cylindrical, and aparaphysate. The ascospores are hyaline, two-celled, generally constricted at the septum and 9 to 13 by 4 to 5  $\mu$  in size. They are subfusoid with subovoid cells, and the distal cells are generally wider than the others. From the morphological characters in the perfect stage, the writer easily recognized the fungus to be a species of *Mycosphaerella* and therefore the name given by Grossenbacher, *Mycosphaerella citrullina* (Smith) Gross., must be used in the future references.

As a control measure the spraying with Bordeaux mixture is recommended in the literature, but the use of this measure soon after we had found the disease in the greenhouse was not successful, probably owing to the fact that the infections had already taken place before that time.

<sup>1</sup> Soon after the manuscript had been prepared, the writer, who was then staying in the United States, received an interesting letter from Mr. Kikujii Kuwatsuka, Plant Pathologist of the Shizuoka Agricultural Experiment Station in Japan. He informed the writer that he had received diseased vines and leaves of the cucumber and of a kind of gourd (*Lagenaria vulgaris* Ser. var. *gourda* Ser.) from Mihomura in the Shizuoka Prefecture and had found on them the fungus, *Mycosphaerella citrullina* (Smith) Gross. The writer has had no opportunity to examine the specimens himself, but this report of the discovery of the fungus on cucumber vines and leaves seems to him to be a valuable contribution to the knowledge of its pathogenicity.

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## PHYTOPATHOLOGICAL NOTES

### *Occurrence of Thielaviopsis paradoxa on the cocoanut palm in Florida.*—

In January, 1922, sections of a diseased trunk of cocoanut palm (*Cocos nucifera*) from Cocoanut Grove, Dade County, Florida, were submitted to the Office of Fruit Disease Investigations through the courtesy of Messrs. G. F. Moznette of the U. S. Bureau of Entomology, and Edward Simmonds, of the U. S. Bureau of Plant Industry. The specimens showed extensive rotting of the ground tissue of the mature parts of the trunk, resulting in internal cavities of considerable extent. Trunk bleeding was reported not to have occurred in the standing tree, which was the only one affected out of a small ornamental planting. The disintegrated tissue when seen by the writer was dry and brittle. Surrounding this was a zone of recently invaded tissue which was brown and water-soaked. Cultures from the advancing margins of the decaying areas, made from tissue removed with aseptic precautions, yielded practically pure cultures of a fungus which agrees closely with published descriptions of *Thielaviopsis paradoxa* (De Seyn.) V. Höhn. To further confirm the identity of the fungus, two firm pineapple fruits from the market were inoculated from pure cultures of the fungus at a number of points through punctures, and a similar number of uninoculated check punctures were made on other portions of these fruits as well as on a third uninoculated fruit. Typical *Thielaviopsis* pineapple rot developed promptly at all the inoculated punctures and no rot developed at the check punctures. A greenhouse plant of Red Spanish pineapple was successfully infected through leaf punctures. *Thielaviopsis paradoxa* was readily recovered in pure culture from the artificially infected pineapple fruits and leaves.

The rate of growth of the fungus was observed in a preliminary series in corn meal agar cultures at maintained temperatures of 10, 15, 20, 25 and 30° C., sowings being made from microspores.

Macrospore formation gave a dense black appearance in two days to the culture at 30° C. A similar appearance was reached in three days at 25° C., and in four days at 20° C. At 15° C. the first sporulation was observed in five days and it did not reach the dense black stage for ten days. Mycelial growth seemed about equally rapid for the first three days at 30° and at 25° C. On the fifth day the 25° C. culture was distinctly in the lead; by the seventh day it had been equaled by the slower

growing culture at 20° C., both of these then exceeding the cultures at 30° C. and at 15° C. By the tenth day the culture at 15° C. had caught up with the one at 30° C. in extent of mycelium growth, both of these being then somewhat behind the cultures at 25 and at 20° C. No mycelium growth was evident during 12 days at 10° C. The indications are that around 25° C. is the best temperature for mycelium development of the fungus. Five degrees above this there seems to be about the same rapidity of development for a short time, followed by a slowing of rate of increase. Five degrees below this assumed optimum growth is somewhat slower, but eventually an equal maximum development is attained. At 15° C. there is very distinct retardation, and a 10° C. visible development does not occur during a twelve-day period.—H. R. FULTON.

*Photographing tube cultures.*—In the literature of plant pathology and bacteriology one frequently sees photographs of cultures that are of little value for scientific purposes because of the “high-lights,” or streaks of reflected light, on the sides of the tubes. Light striking this curved surface is reflected in all directions; and a reflection shows in the picture for each source of light. Some rather complicated methods have been described for the prevention of these objectionable features; but they can be avoided with the ordinary photographic equipment by a little care in placing the tube. For those who have not already solved the problem the following may be useful:

(1) *Use light from only one direction.* With natural light it is best to use only one window. (2) *Place the tube so that its long axis lies within the plane formed by the tube, the source of light and the camera;* only light reflected in this plane can now show in the photograph. This reduces the reflecting surface of the tube, so far as the picture is concerned from a curved mirror to a flat one. If the tube is perpendicular to the camera, as it should be to obtain proper perspective, no streaks of light will now show unless the angle, light to culture tube to camera, is very acute. Widen this angle if necessary by tilting the camera and tube, or by moving the camera and tube farther from the window, or if using artificial light by moving the light.—WALTER N. EZEKIEL.

*Variation in color of pear blight exudate.*—While carrying out an experiment with pear blight, a peculiarity in color of the exudate was observed. Several loquat trees, (*Eriobotrya japonica* Lind.) were inoculated with a pure culture of *Bacillus amylovorus*, (Burrill) De T., in bouillon by means of the hypodermic needle, March 20, 1921. These inoculations were made in the growing tips of young trees in the green-



house. Five days after the inoculation beads of exudate appeared. The exudate was mostly of the characteristic colors. However, on one twig there were several good sized beads of a dark green color, not compared with color chart but approximating Ridgway's Meadow Green. The color did not fade nor change with standing. On examination it was found that the green exudate was filled with bacteria which agreed with the pure culture of *B. amylovorus* as to staining reactions and general morphology. Later this particular twig showed the characteristic wilt and blight the same as shown by twigs producing the usual form of exudate. The later beads did not show the green color.

It is evident that the green exudate is very rare as no note of its occurrence has been found in literature.—J. P. MARTIN, Laboratory of Plant Pathology, University of California, Berkeley, Cal.

*Personals:* Dr. R. D. Rands, for the past three years engaged in rubber disease research for the Dutch government at Buitenzorg, Java, has recently returned to this country and accepted an appointment as pathologist in the Office of Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C. Doctor Rands will take charge of the Department's work on diseases of beans, with headquarters in Washington.

During the summer Messrs. C. O. Peak, O. A. Plunkett, C. L. Porter and P. A. Young will be employed in Plant Disease Survey work in the State of Illinois. This survey is under the general direction of F. L. Stevens and under the special direction of Mr. L. R. Tehon.

Dr. A. G. Johnson, Associate Professor of Plant Pathology, University of Wisconsin, and Pathologist, Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, formerly stationed at Madison, Wisconsin, has transferred headquarters to Washington, D. C., where he will continue his work in the Office of Cereal Investigations. He has resigned his University appointment.

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# PHYTOPATHOLOGY

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## STEM AND FRUIT BLIGHT OF PEPPERS CAUSED BY PHYTOPHTHORA CAPSICI SP. NOV.<sup>1</sup>

LEON H. LEONIAN

WITH TWO FIGURES IN THE TEXT

In the fall of 1918 the writer found that a disease was causing considerable damage to the Chile pepper plants on the farm of the New Mexico Experiment Station. A preliminary examination revealed nothing more than an *Alternaria*, and although this fungus was isolated readily, repeated inoculation experiments with it gave only negative results. It was concluded, therefore, that this organism was merely a secondary invader that followed the injury caused by the primary infecting agent. A year later the same disease reappeared in the field and assumed quite serious proportions. Other fields in the neighborhood were found to be generally affected with it. This time isolations were made from very young lesions. After the surface of the infected tissues was carefully washed with a mercuric chloride solution, flamed forceps were inserted beneath the epidermis, just on the border of diseased and healthy regions, and a small bit of tissues was removed and planted in a poured plate of cornmeal agar. The fungus which eventually grew out of the diseased material was found to be a species of *Phytophthora*, and when pepper plants were inoculated with it 100 per cent infection was the invariable result.

### THE DISEASE

This disease appears on the branches as a blight, and on the pods as dry lesions of various sizes. If not followed by an attack of *Alternaria* the market value of the diseased pods is not impaired. When the *Alternaria* attack is not severe, only an experienced observer can detect any external indication of the disintegrated tissues beneath the epidermis and signs of blackish mouse-gray mycelium of the *Alternaria* that stuffs the inside of the pod.

<sup>1</sup> The writer is indebted to Dr. C. H. Kauffman for the critical reading of the manuscript of this paper.

The disease generally appears anytime after May when the warm and rainy season begins, and continues until late fall. The first indication of it on the pod is a small, water-soaked, dull green spot which in mild cases does not enlarge very much. In more severe cases elongated lesions which eventually may involve one fourth or more of the pod develop. Very rarely does a natural infection extend throughout the fruit, and in no case has the writer observed a lesion which encircled the pod like a band. The advance of the infection is much more rapid along the stem-end and blossom-tip of the pod than it is along its circumference. A natural infection by this organism is not indefinitely progressive, but for some unexplained reason it eventually comes to a sudden halt; consequently the lesions are narrow, long, and sometimes extend the entire length of the pod. The infected tissues soon dry, bleach to a dull straw color, sink below the level of healthy tissues, and become parchment-like (Fig. 1). The seeds also become infected; then they turn brown, shrivel, and in severe cases die; or they may retain their vitality if the infection is confined to the seed coat only.



FIG. 1. Tip of a Pepper Fruit Showing a Typical Lesion.

Often the fungus passes from the fruit to the branches; then it grows rapidly and involves the younger and tender parts. However, it often extends to the larger branches which are completely girdled and finally killed. Usually after the fungus reaches the older parts or the main stem, its growth stops, so that frequently only one branch, or one half, or even one third of the plant is killed, while the remaining part stays healthy. More frequently the branches become infected not through the fruit but directly at their base by means of the zoospores of the pathogen. A ring of blighted tissues then appears, and the parts above

it soon wilt. If the primary infection is on the main stem, the entire plant is killed; but if it starts on a branch, it moves downward, and either before or after it reaches the main stem, its growth ceases. It is an interesting phenomenon that the infection on the branches progresses around rather than up and down in narrow lesions as is true in the case of infection of the fruit.

#### THE FUNGUS

The fungus responsible for the disease of Chile peppers is a species of *Phytophthora*, and according to the classification of Rosenbaum (3) it falls in the *Phaseoli* group because of its basal antheridium. A morphological comparison with the other members of the group shows that this fungus is apparently a new species for which the name of *Phytophthora capsici* is proposed. The gnarled mycelium of this fungus is very distinctive. Of all the eleven species of *Phytophthora* studied by Rosenbaum (3), *P. syringae* is the only one which produced a "tuberculate" growth on potato hard agar. *P. syringae*, however, in addition to its small zoosporangia, forms the antheridia on the side of the oogonia, whereas *P. capsici* has very much larger zoosporangia, and basal antheridia. *Phytophthora parasitica*, *P. phaseoli*, and *P. infestans* of the *Phaseoli* group can also be eliminated because of their smooth hyphae when grown on potato hard agar, the much smaller size of their zoosporangia, and because of the lack of ready oospore formation in case of *P. infestans*. Of the remaining two organisms *P. erythrosepatica* has a broad and flat papilla in contrast to the raised and very prominent papilla of the zoosporangia of *P. capsici*. The last member of the *Phaseoli* group as given by Rosenbaum, *P. arecae*, forms a characteristic vesicle into which the zoospores escape. No such vesicle is seen in *P. capsici* upon the germination of its zoosporangia. Two apparently new species of the *Phaseoli* group have been reported recently. One of these affects the rhubarb, and the other causes a rot of the tomato fruit. But since these organisms still remain undescribed, no comparison is possible at present.

#### *P. capsici* sp. nov.

Sporangiophores branched; sporangia generally ovoid, varying in culture to elongated ellipsoid, subsphaeroid, irregularly elongate with intermediate forms; papilla very prominent, either single and apical, or sometimes up to three and variously disposed; germination normally by zoospores, under special conditions by germ tubes; size of sporangia extremely variable, 35–85  $\mu$  or even 105 x 21–56  $\mu$ , averaging 60 x 36  $\mu$ ; oospores formed on submerged mycelium, abundant on oatmeal and corn-

meal agars, slightly wrinkled, brown, semi-transparent, 25–35  $\mu$ ; antheridia basal; mycelium often gnarled, becoming densely tuberous under certain cultural conditions; the tuberous outgrowths sphaerical or ovoid, minute, 5–8  $\mu$ , of the same shape as sporangia, richer in protoplasm and darker in color than the mycelium, often very numerous, giving rise to large, grape-like clusters, germinating by germ tubes; no chlamydospores observed. Parasitic on stems and fruit of *Capsicum annuum*. State College, N. Mex.

The characteristic tuberous outgrowths (Fig. 2, c) appear on a great many media, potato hard agar included, but they attain their maximum growth in a solution<sup>1</sup> which has been developed and used by the writer in connection with some other investigations. Only very few sporangia are formed in this solution, but when the mycelium is transferred from this medium to either distilled water or to some nutrient solution which induces sporangia formation, these tuberous bodies germinate by a germ tube which elongates into a sporangiophore, and gives rise to a sporangium (Fig. 2, d). When transferred to distilled water, the protoplasm of the mycelium, as well as that of a number of these outgrowths, migrates into the sporangiophores and becomes separated from the empty hyphae by one or more cross walls. This, however, is not the case when the hyphae are transferred to a favorable nutrient solution; here a normal growth precedes the sporangia formation. These tuberous outgrowths must be considered as a distinct morphological characteristic of this species because of their regular occurrence on a wide variety of nutrient media. Similar bodies have been observed by Ward (5) in *Pythium gracile*. Butler (1) found "toruloid structures" in *Pythium*. Rosenbaum (3) reported the "gnarled and tuberculate" appearance of the mycelium of *Phytophthora syringae* when this fungus was grown on potato agar, and he used this characteristic to separate *P. syringae* from all of the other species which he studied.

#### INOCULATION EXPERIMENTS

Inoculation work with this fungus has covered a period of three years. Field work was done during the summer of 1920 by the writer, and by Mr. W. A. Archer during the summer of 1921. Greenhouse inoculations have also been made by the writer throughout 1921 and 1922 at Ann Arbor, Michigan. The results have been uniformly positive. The fungus is capable of attacking the host through sound tissues. Under the climatic conditions of New Mexico the first visible lesions on the

<sup>1</sup>  $\text{KH}_2\text{PO}_4$  1. 2 grams,  $\text{MgSO}_4$  0.6 gram, peptone 0.6 gram, maltose 6.25 grams, malt extract 6.25 grams, distilled water 1000 cc.

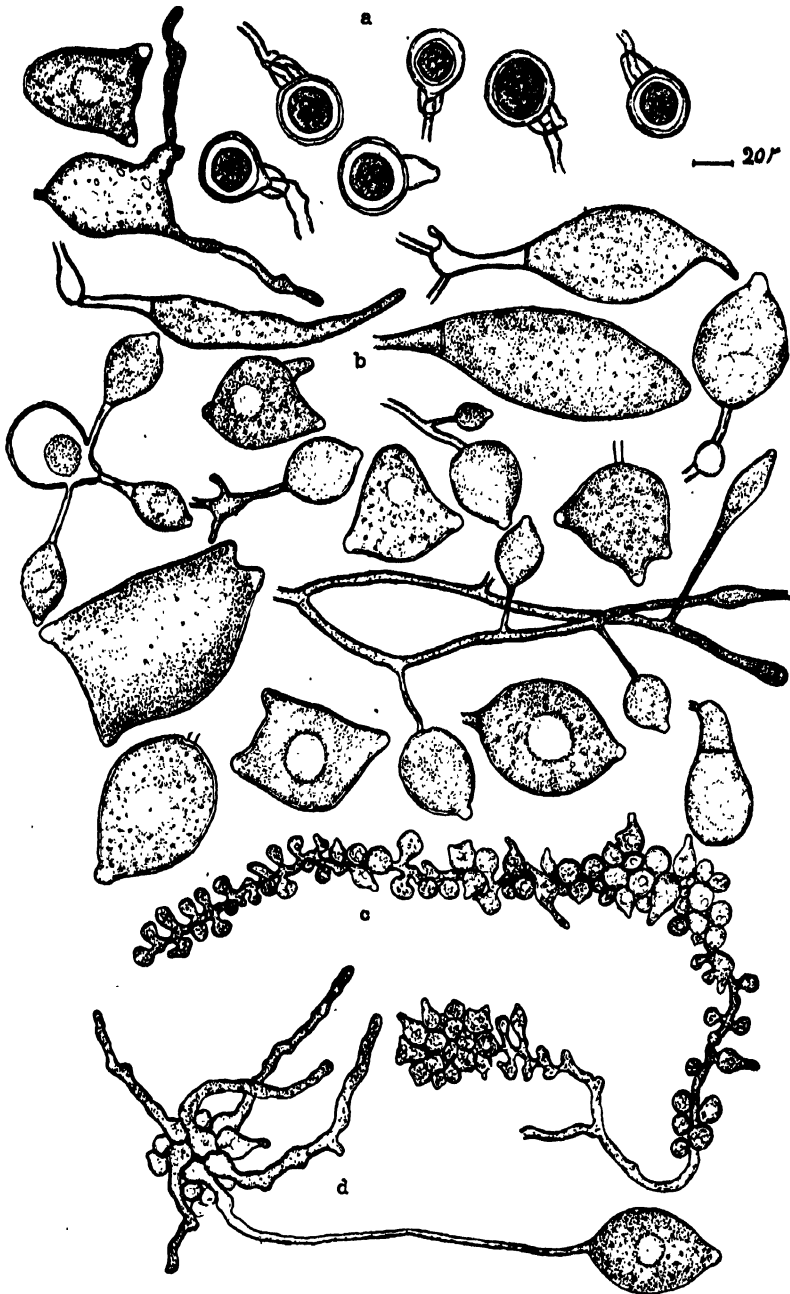


FIG. 2. *Phytophthora capsici* sp. nov. (a) Oospores. (b) Sporangia; Different Types and Stages of Development. (c) Tuberos Outgrowths Formed on the Mycelium. (d) The Tuberos Outgrowths Germinating; One of Them has given rise to a Sporangium.

fruit appear within 24 hours after inoculation and within a period of three days most of the pod becomes infected. Shortly thereafter no sound tissue remains on the pod which is at first soft and pulpy, but gradually dries and shrivels. Meanwhile, if the drying does not proceed too rapidly, the sporangial masses are borne on the surface of the pods within the folds of the wrinkled tissues. Just why infection should progress so rapidly in the artificially inoculated fruits, and why it should advance so slowly and restrict itself to limited areas in case of natural infection, is not very clear, unless it be assumed that mass action plays an important rôle. Bits of mycelium containing a number of sporangia have been used as inoculum in the artificial inoculation experiments, while in case of natural infections it is most likely that only a single zoospore constituted the original inoculum. Smith (4) states that mass action plays an important rôle in the infection brought about by some bacterial pathogens. Heald (2) observed that in artificial smutting at least 0.5 gram of powdered smut well distributed to each 100 grams of seeds is necessary to produce maximum smutting, and that with small spore loads certain varieties of spring wheat remained smut-free. This indicates that either a multiple infection or mass action by the spores is the cause of this phenomenon.

The first indication of the disease on the woody stems can be seen within a week from the time of inoculation. At first a brownish discoloration appears which gradually enlarges, and in another week a dark brown band of tissues girdles the stem. On the tips of younger branches, however, the infection manifests itself within 24 hours. Then the fungus moves upward and downward, passes from branch to branch, and finally ceases to make further progress in spite of the most favorable environmental conditions. In case of greatly under-nourished and weakened plants in the field, and of pot-bound, sickly looking plants in the greenhouse there is no arrest of the disease, the entire plant, roots and all, being invaded by the pathogen and killed.

Under ordinary field and greenhouse conditions the pathogen does not cause a root rot. Repeated inoculations of the soil in which the plants were growing, gave negative results. When diseased plants were buried in the soil in direct contact with the roots of healthy plants, no losses occurred. When after a time these plants were dug out and examined, only a few of the roots were found to be diseased, and it was noticed that the infection, after having made some headway, had stopped, just as in the case of the branches. In another experiment some of the roots of a healthy plant were exposed, inoculated with the mycelium of the pathogen, and again buried in the soil. Later when these were

examined, the disease was found to have progressed from the tips of the finer roots to the larger ones, and then to have come to a sudden halt. This strange phenomenon apparently is not due to the woody nature of the tissues alone, nor is it caused by the death or by the loss of the physiological efficiency of the pathogen, but apparently is due to a local immunity presented by the host. It was noticed that on the branches, after the infection had stopped its progress, the live tissues which immediately adjoined the killed ones had assumed a dark purplish color. In one experiment when this dark region was used as a court of infection, even when it was wounded and stuffed with the mycelium of the pathogen and kept in a saturated atmosphere, no infection occurred. Three attempts at different intervals gave similar results. When, however, the tissues just below this dark region were inoculated, infection resulted and in due time the plant was killed. The immunity, the toxic agent, or whatever term we might choose to apply to this inhibiting factor which is developed only by vigorously growing plants, is highly localized and does not diffuse in the tissues. It is also probable that structural changes, caused by the action of the fungus upon the plant, are responsible for this local immunity.

#### DISSEMINATION

The seeds located just beneath the diseased lesions of the pods are seen in various stages of infection. Some are brown and badly shriveled; others may or may not show very distinct external signs of the disease, but when they are planted in nutrient agar they give rise to the mycelium of the pathogen. Some of these seeds when planted in agar germinated in spite of the fungus which was growing out from the seed coat. The young sprouts, however, were soon destroyed by the hyphae. Thus it becomes apparent that infected seeds constitute important agents of dissemination for the fungus. Diseased pods, as a rule, are not discarded by the growers because, as stated before, their diseased condition is hardly noticeable externally. Since the infection invariably extends to the seeds, it follows that if the diseased material finds its way into hot beds, cold frames, or the field, the pathogen immediately starts growing. It may attack some of the roots, without, however, killing the young plants, or it may be carried to the field with the infected soil which clings to the roots. Thus new centers of infection form. Once established in the soil the pathogen may become very difficult to eradicate. It is a matter of common observation that the pods situated on the lower branches are the ones to become diseased, and that the initial infection, at least in the majority of cases, starts near the blossom end.



Since the tip of the fruit usually hangs down, the blossom-end is in the path of the zoospores which are forced up by spattering rain drops. Similarly stem blight usually start at the lower parts of the branches or stems where the zoospores are most likely to find a favorable lodging.

#### CONTROL

In view of the foregoing facts the chief control measure should consist in careful seed selection especially if the fungus is not already established in the field. In case the soil is already infected, spraying with fungicides is recommended. While the writer has tried no spraying experiments, it is obvious that zoospores formed in the soil and carried up to the plants are responsible for the infection. A careful spraying schedule, if followed properly, should not fail to give good results.

#### SUMMARY

1. A disease which attacks the pods and the branches of Chile peppers in New Mexico was found to be caused by a new species of *Phytophthora*.

2. The disease manifests itself in the form of dry lesions on the pod. The younger branches are killed outright, while on the older parts of the hosts the blighting of the tissues brings about a rapid girdling.

3. The disease is not indefinitely progressive; the infection stops after reaching the older parts. Artificial inoculations of the sound tissues at the point where the disease stopped, failed to cause infection, while the region a little below this point failed to exhibit any resistance to the pathogen.

4. The fungus can only affect a few of the younger roots.

5. The seeds in the pods become infected and thus serve to disseminate the organism. When infection is confined to the seed coat, the power of germination is not interfered with.

6. Seed selection and spraying with fungicides are recommended as control measures.

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# THE INFLUENCE OF SOIL TEMPERATURE UPON THE DEVELOPMENT OF FLAX WILT

L. R. JONES AND W. B. TISDALE

WITH ONE FIGURE IN THE TEXT

In 1914-16, W. H. Tisdale working at the University of Wisconsin (4) made a study of the nature and inheritance of resistance in flax to the wilt disease caused by *Fusarium lini* Bolley. While growing plants in the greenhouse for these experiments he (5) noted marked differences in the rate of wilting of susceptible flax plants growing at different distances from the heating system. Upon investigation, the temperature of the soil nearest the steam pipes was found to be several degrees higher than that more remote from the pipes.

Following out the suggestion that soil temperature might be an important factor influencing the rate of infection, Tisdale devised a simple apparatus for holding the temperature of the soil below that of the room. From trials made with this he reached the conclusion that the critical temperature for the infection of flax by *Fusarium lini* is between 14° and 16° C. The disease became serious with plants in control pots with temperatures at 19° to 21° C. Because of the lack at that time of adequate apparatus for maintaining constant soil temperatures, the minimum temperature for occurrence of the disease was not exactly determined and no effort was made to learn either the optimum or maximum temperature limits for disease development. Following the perfection of the Wisconsin soil temperature tanks (2), the writers during 1921 extended the studies upon the disease through a wider range of soil temperatures, and the (2) data obtained are here presented.

The flax seed used was the susceptible strain, C. I. No. 3, furnished by Mr. J. C. Brinsmade of the Office of Cereal Investigations, U. S. Department of Agriculture. The soil was obtained from a well drained marsh field at Madison which had previously been successfully inoculated with a pure culture of the flax *Fusarium*. This originally came from the North Dakota material furnished by Professor H. L. Bolley. The water-holding capacity of this soil was 67 per cent (40 per cent based on wet weight) and the moisture content was kept at 25 per cent during the experiments, a condition favorable for the flax plant. A uniform amount of the soil was placed in each culture can (8 x 12 inches), and those to be used as controls were steamed for five hours at one or two

pounds pressure and allowed to stand for a week before planting. After they had been in the tanks for a sufficiently long period for the temperature to become adjusted, one hundred flax seeds were planted in each can. The experimental soil temperatures ranged in a graduated series from 12° to 38° C. with 4° or 3° intervals.

The first trial was conducted in April and it was repeated in May with essentially like results. These are combined in table 1.

TABLE 1

*The percentage of flax seedlings developing wilt at different soil temperatures. Based on two experiments in each of which the plants were grown for 24 days.*

TEMPERATURE OF SOIL (°C.)	TOTAL NUMBER PLANTS	TOTAL NUMBER PLANTS WILTED	PERCENTAGE OF WILT
12	170	0	0
16	153	48	31
20	154	104	68
24	151	147	97
28	129	123	95
31	129	103	80
34	100	67	67
38	36	0	0

Since so high a percentage of wilt developed at 16° C. it seemed probable that the critical temperature was distinctly below this. In order to learn this more definitely a third experimental series was run in the same way limited to two soil temperatures, the first 14° to 15° and the second 16° C. The results were that at 16° C., ten plants out of 45 plants, or 22 per cent, wilted within the 24 day period. This was in essential agreement with the results from the first two trials. At 14° to 15° C., however, out of 40 plants only a single one showed slight symptoms of wilt in the same period. The lower critical soil temperature for disease development seems therefore to be about 14° C. The delicacy of the temperature balance was further shown when at the end of the above trial period of 24 days the pots which had been held at 14° to 15° were raised to 16° C. At the end of one week 15 per cent of these plants showed wilt symptoms.

These results are graphed in figure 1. They are in general accord with those of W. H. Tisdale (5) at the lower range of temperatures and add interesting data concerning developments at the higher range. It is noteworthy that in this case as with the other vascular Fusarial diseases tested in the Wisconsin soil temperature tanks (6, 1) the disease is almost as sharply limited in its occurrence at the upper temperature range as at

the lower (see table 1 and figure 1). A comparison of the temperature range of the disease with W. H. Tisdale's results with pure cultures of *Fusarium lini* on agar plates (5) shows that this *Fusarium* flax wilt disease behaves much as does the *Fusarium* cabbage yellows disease. Flax wilt developed at the highest temperature (34° C.) at which the parasite grows in pure culture on agar. The optimum temperature for the disease development practically coincides with the optimum for the cultures of the fungus (24° to 28° C.). At the lower temperature the growth of the fungus may occur at a slightly lower temperature (12° to 13° C.) than the disease development (14° to 15° C.).

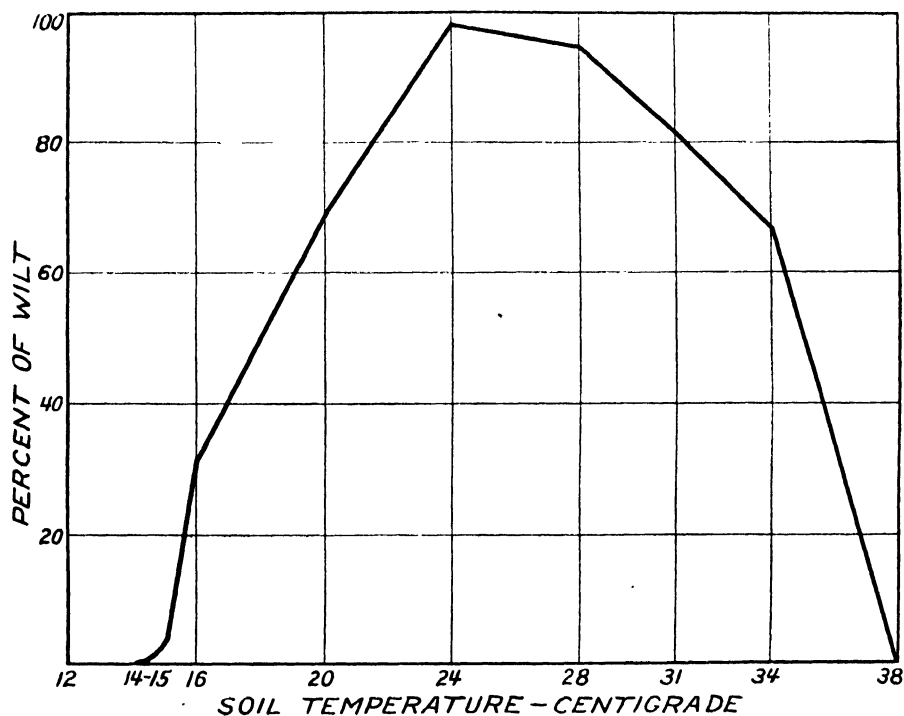


FIG. 1. Graph showing the per cent of wilt developing in flax seedlings at different soil temperatures. For details see table I and the accompanying text. Note that the minimum or "critical temperature" for disease development is about 14 degrees, the maximum apparently 38 degrees or below and the optimum for extreme wilt development between 24 and 28 degrees C.

It is of further interest to note that this is considerably lower than the corresponding lower critical temperature for the occurrence of the tomato wilt disease, caused by *Fusarium lycopersici*, which Clayton (1) has shown to be about 19° C. It is also somewhat lower than that for the cabbage yellows, caused by *Fusarium conglutinans*, which Gilman and W. B. Tisdale define as about 16° to 17° C. The correlation of these

critical temperatures seems to be closer with the temperature relations of the normal host than with those of the corresponding fungi in pure culture on agar. Thus while all these *Fusarium* species show similar pure culture temperature relations it is evident that the tomato is normally a higher temperature plant than cabbage or flax. It is also noteworthy that these lower temperature ranges as defined by the present experimental method bear a relation to each other which corresponds in a general way to the distribution of these diseases in the northern Mississippi valley. Thus the tomato wilt finds its northern limit of aggressive field development south of the latitude of Chicago; the cabbage yellows, while at its worst in the second tier of states (Indiana, Illinois, Iowa), reaches into southern Wisconsin and Minnesota; the flax wilt is serious to the northern limits of North Dakota. Stoa's (3) discussion of conditions in North Dakota indicate that the average temperature for the month of July at the North Dakota Agricultural College, the hottest month of their summer, practically coincides with this optimum temperature for disease development. Stoa notes with flax wilt, as has been noted with cabbage yellows in Wisconsin, that the disease is particularly destructive during certain seasons when midsummer temperature is high, and is less severe during cool summers.

#### SUMMARY AND CONCLUSIONS

(1) Flax wilt caused by *Fusarium lini* is readily influenced in its development by soil temperature as was first shown by W. H. Tisdale.

(2) From his work, using only the lower soil temperatures of limited range, he concluded that the critical low temperature for infection lay between 14° and 16° C. He did not determine the relation to higher temperatures.

(3) The present experiments using the Wisconsin tank method included trial cultures at soil temperatures of approximately 12, 14, 16, 20, 24, 28, 31, 34, and 38 degrees C.

(4) The disease was entirely inhibited at both the lowest and the highest of these temperatures. The minimum or "critical" soil temperature for the disease is about 14°, with 38° C. as the maximum, and the optimum for extreme disease development about 24° to 28° C.

(5) The flax plant will develop in *Fusarium* infested soil without wilting at both the higher and the lower temperature extremes. Germination is retarded at 12°, but once started the plants make a good stocky growth. At 38°, the growth is weakly. The best normal vegetative development occurs in the neighborhood of 20°, that is, distinctly below the optimum for the wilt.

(6) The temperature curve for the disease corresponds closely with

that for the growth of the parasite. The optimum for disease development, 24° to 28° C., is at the same time the apparent optimum for the vegetative development of the fungus in agar culture.

(7) The field evidence as to the distribution and seasonal variation in severity of the disease is in harmony with these experimental results since the evidence for North Dakota where the disease occurs indicates that flax wilt is aggravated by hot seasons and that their midsummer temperature corresponds closely with the optimum for the wilt.

(8) The data are now available for a comparison of the temperature relations of the very similar *Fusarium* diseases of the flax, cabbage, and tomato. The behavior of the respective parasites in pure cultures on agar is practically identical. On the other hand the host plants have quite different temperature relations, the tomato being favored by distinctly higher temperatures than either of the others, and, as between the latter, flax seems to be a slightly cooler temperature plant than cabbage. It is possible that there is a natural correlation between this host relation and the fact that the lower or "critical temperatures" for the occurrence of their respective diseases stands in like sequence, that of tomato being about 19°, of cabbage about 16° to 17°, and of flax about 14° C.

(9) The geographical distribution of these diseases in the Mississippi valley also seems to be influenced by these temperature relations. The tomato wilt is a distinctly southern disease with its northern range in the latitude of central Illinois; cabbage yellows is at its worst in the second tier of northern states (Ohio to Iowa) with its northern limit in the latitude of southern Wisconsin; flax wilt reaches to the northern boundary of the United States and probably into Canada.

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# A PHOMOPSIS IN GRAPE FRUIT FROM THE ISLE OF PINES W. I., WITH NOTES ON DIPLODIA NATALENSIS

WM. TITUS HORNE

WITH PLATES XXVI AND XXVII AND ONE FIGURE IN THE TEXT

On October 30, 1917, Mr. Frederick Maskew, in charge of plant quarantine work for the State Commission of Horticulture at San Francisco, sent several grape fruits to the Division of Plant Pathology at Berkeley for advice as to whether certain spots (Pl. XXVI, fig. 4) should be classed as melanose (12, 6, 9, 7, 10). The fruits were from a shipment said to come from the Isle of Pines, W. I., the fruit wrappers bearing the mark "Isle of Pines Grape Fruit, S. A. Girard & Company, Sole Distributors."

The fruit was of excellent quality and, aside from the spots in question, had not more than the anticipated blemishes for West Indian grape fruit. These blemishes are treated in a separate paper (11).

The spots were small red brown dots rather evenly scattered, mostly on the blossom end of one fruit. In mature fruits the causal fungus of melanose, *Phomopsis citri* Fawcett, is believed to have died out, and accordingly no attempt was made to study this fruit farther. As far as known *P. citri* had not been reported from the Isle of Pines or Cuba.

On the following day, examination of the suspected grape fruit shipment was made with Mr. Whitney of Mr. Maskew's office. A number of additional specimens were taken including two fruits with a tan-colored rot. These fruits were numbered 6 and 7 and were put in moist chambers in our laboratory for study. Cultures were made from them and kept until the summer of 1918 when they were dried out.

Number 6 was a well developed specimen of Diplodia rot (3, 7; 1, 2) apparently little changed except in color, the clear yellow replaced by tan with some dark shadings and the surface gloss unbroken. Number 7 was in a much earlier stage of rot, only part of the surface being involved, but not certainly distinguishable from 6. Both fruits were kept in moist chambers and various tissue plant cultures made from them. Number 6 gave cultures of a fungus which was evidently *Diplodia natalensis* I. B. Pole-Evans, and readily produced stem end rot (4) in grape fruit and oranges on inoculation in the laboratory (Pl. XXVI, figs. 2, 3 and Pl. XXVII, figs. 7, 8, and 11).

Number 7 gave no culture of *Diplodia* but a more delicate white fungus, a *Phomopsis*, (5, 6, 8) which appeared regularly but was frequently overcome by contaminations, especially in media rich in nutrients (Fig. 1, a and b, and Pl. XXVII, figs. 9, 10, 12 and 13). In the later stages shadings of a smoky uniform brown appeared below the surface but not the black subsurface color shown by 6. When in the early stages of deterioration number 7 was cut in halves and left in the moist chamber. White mycelium appeared generally over the cut surfaces but there was no blackening as occurs with *Diplodia* mycelium.

#### CULTURE CHARACTERS OF THE PHOMOPSIS.

On slant tubes of agar with very little nutrients the growth is very delicate, colorless, becoming barely brown tinted, filling the medium with a little white aerial mycelium at the top and a few scattered dark brown sclerotial bodies less than 0.5 mm. in diameter.

On nutrient agar in slant tubes the surface is covered with a fine white matted mycelium. Dark brown bodies occur, and a general brownish somewhat flesh colored pigment spreads into the medium (Pl. XXVII, fig. 9).

On green orange twigs flamed quickly and dropped into sterile water so as to leave about 2 cm. of twig above the liquid, growth was excellent. Cut surfaces become covered with white matted cottony mycelium, the unbroken epidermis becomes brownish black, lightly over-spread with fine appressed mycelium, and broken by numerous small conical pycnidia evenly scattered over the whole surface (Pl. XXVII, fig. 10). As the liquid dries successive interrupted pellicles are formed. They are white to yellowish and smoky brown. There is apparently no growth in the liquid which becomes greenish amber. Some dirty white beads form on the lower pycnidia. Lower pycnidia (newer and moister) have the scolecospores or paraphyses predominating, 20 to 32 by  $1\ \mu$ , (Fig. 1, b). Upper pycnidia have mainly spores 5.6 to 8 by 2.2 to  $3\ \mu$  (Fig. 1, a).

The fungus was grown on a number of media without difficulty. Slight alkalinity causes it to form a small and weak colony (Pl. XXVII, fig. 12) while slight acidity is not detrimental (Pl. XXVII, fig. 13). Limits of tolerance were not determined.

#### INOCULATION EXPERIMENTS WITH PHOMOPSIS.

Inoculations were made on grape fruit and typical stem-end rot developed in several specimens (Pl. XXVI, figs. 5 and 6). The fungus was reisolated and grown in culture in the laboratory at room temperature, giving the characteristic features. About three months elapsed in



all cases between the time of inoculation and the development of undoubted stem-end rot. Fruits were kept in moist chambers in the laboratory and many were lost with contaminations. Successful inoculations were made with both mycelium from agar culture and with spores placed on the calyx and on the wounded calyx scar. None of the inoculations on oranges were completely conclusive. Some were probably successful but were interfered with by contaminations. In the late phase of *Phomopsis* rot brownish black points emerged through the surface of the fruit and white mycelium spread from these (Pl. XXVI, fig. 6) covering the whole fruit with a finely felted mycelium in which black bodies were prominent (Pl. XXVI, fig. 5). In some cases straw colored beads appeared on these bodies. The fruit very gradually became soft and collapsed leaving the outer shell the last part to disorganize.

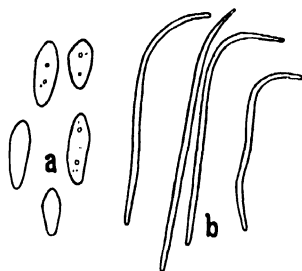


FIG. 1. a. Spores of *Phomopsis* from Isle of Pines from culture on green orange twig,  $\times 1000$ . b. Scolecospores (or paraphyses) from same culture as a.  $\times 1000$ .

#### INOCULATION EXPERIMENTS WITH *DIPLODIA*.

*Diplodia* inoculations were uniformly successful, the stem-end rot well developed in about one month in moist chamber in the laboratory. In later phases of *Diplodia* rot considerable subsurface black color developed and strong mycelium tufts grew out and later spread over the fruit in an undulating coarse cottony cover which became black except the outermost hyphae which remained gray (Pl. XXVI, fig. 2). Conspicuous sclerotial bodies up to 1 cm. tall and 0.5 cm. broad appeared in some cases (Pl. XXVI, fig. 3).

Most of the culture work reported is based on homogeneous and not single spore cultures and was intended as preliminary work only. While it would be desirable to repeat many of the details of this study it seems better to give out the results than to wait until the opportunity comes to

secure more of this material from the Isle of Pines. The fungus appears sufficiently distinct to be recognized as a new species and I suggest the following specific name with brief description.

**Phomopsis caribaea** sp. nov. Phomopsis isolated from grape fruit affected with stem-end rot, from the Isle of Pines, W. I. Spores from green orange twig in culture  $5.6-8 \times 2.2-3 \mu$ , scolecospores  $20-32 \times 1 \mu$ . Growth in nutrient agar culture differs from *Ph. citri* Fawcett in being more vigorous and retaining its filamentous aspect, not becoming finely granular or limelike. Causes stem-end rot of grape fruit in artificial inoculation.

Type specimen, a culture similar to that shown in plate XXVII, figure 10, is deposited in the herbarium of the University of California. Cotype material from the same fruit but a different isolation is being sent to the New York Botanical Garden and to the Bureau of Plant Industry, Washington, D. C.

The generous assistance of Dr. H. S. Fawcett in these studies is gladly acknowledged.

#### CONCLUSIONS

A species of Phomopsis was isolated from grape fruit from the Isle of Pines, W. I.

This fungus is capable of producing stem-end rot in grape fruit but under laboratory conditions develops very slowly. It can probably also produce stem-end rot in oranges.

In green orange stems in moist tube culture it develops vigorously.

In nutrient agar culture it appears to differ from *P. citri* Fawcett in being more vigorous and in having the surface of the colony show fine matted filaments instead of a "chalky" surface.

Certain blemishes, tear stains and rots of grape fruit in Cuba and in the Isle of Pines might well be studied with this Phomopsis in mind, as well as deficient moisture or plant food in the soil, exposure, mite and insect injury. The fungus in question is considered to be distinct and the name *Phomopsis caribaea* is suggested for it.

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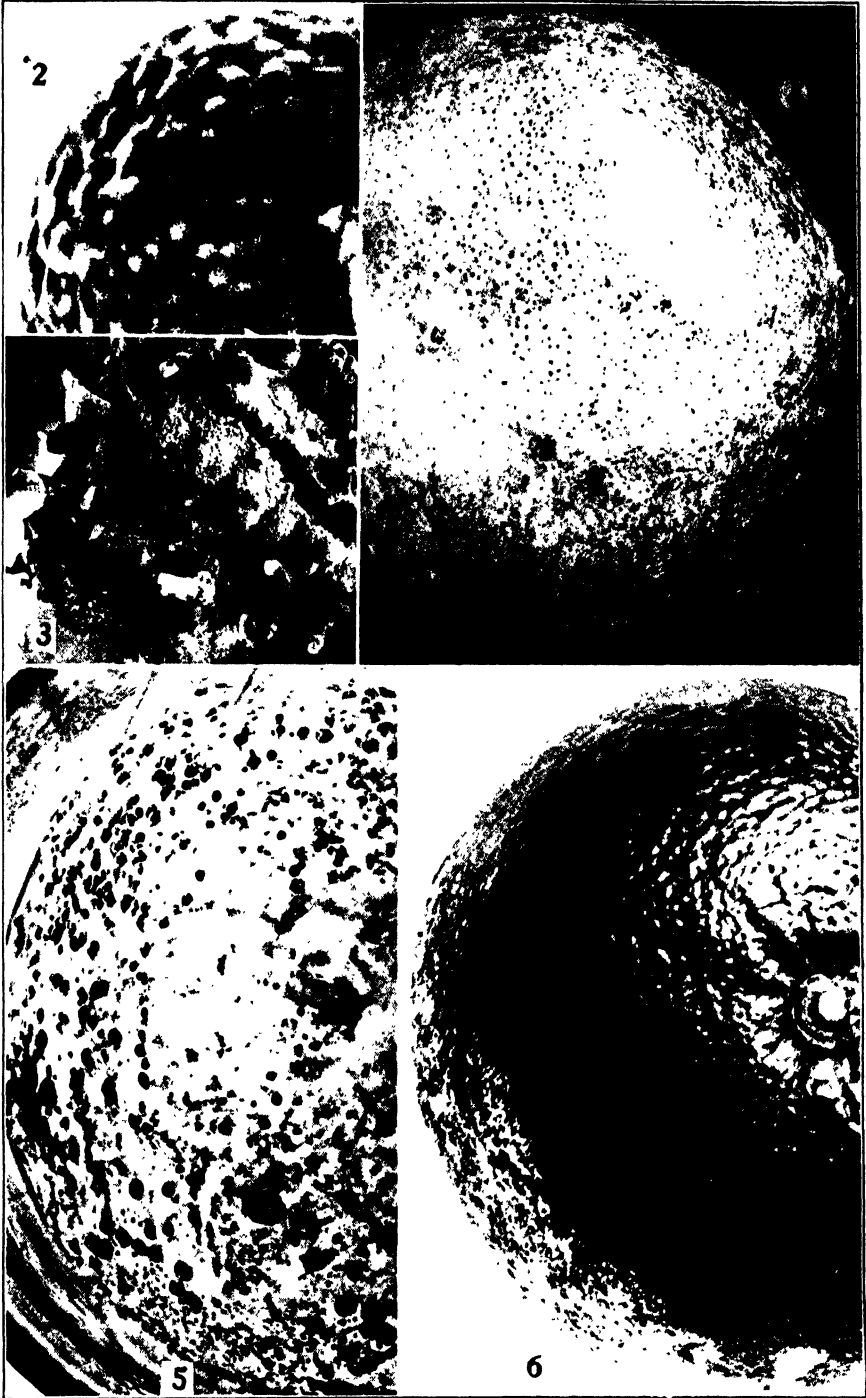
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#### DESCRIPTION OF PLATES XXVI AND XXVII

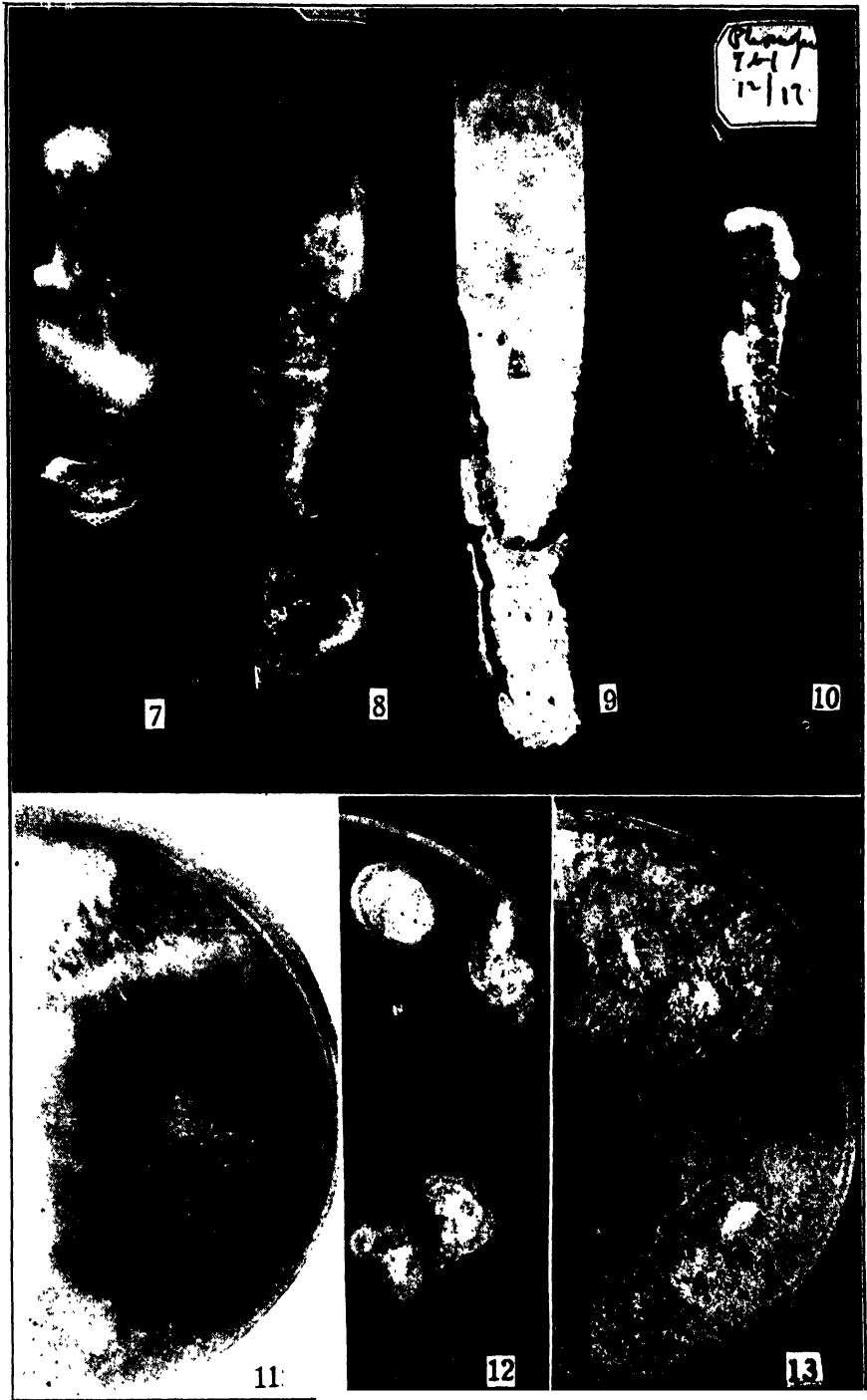
Pl. XXVI (2) Part of orange with *Diplodia* rot in moist chamber after dark mycelium has emerged and covered the fruit. Artificial inoculation with fungus from the Isle of Pines, W. I. (3) Very old phase of *Diplodia* rot in moist chamber showing sclerotial bodies, artificial culture as figure 2 on grape fruit. (4) Grape fruit from the Isle of Pines, W. I., with small spots suspected of being melanose. (5) *Phomopsis* from Isle of Pines inoculated on grape fruit, very old phase of culture. (6) Grape fruit with typical stem-end rot in moist chamber from inoculation with *Phomopsis* from the Isle of Pines fungus tufts are beginning to emerge at the left below, after the fruit is completely invaded and turned a light tan color.

Pl. XXVII (7) Culture of *Diplodia* from Isle of Pines on green orange twig. (8) As 7 on nutrient agar slant. (9) *Phomopsis* from Isle of Pines culture on nutrient agar slant. (10) As figure 9, culture on green orange twig. (11) Reverse of a petri dish culture of *Diplodia* from Isle of Pines in nutrient agar. Photograph pale, the black color is very pronounced. (12) As figure 9, culture in nutrient agar made somewhat alkaline in petri dish. (13) As figure 12, but medium somewhat acid.



PHOMOPSIS OF GRAPEFRUIT.





PHOMOPSIS OF GRAPEFRUIT.



# A NEW PHOMOPSIS OF CITRUS IN CALIFORNIA<sup>1</sup>

HOWARD S. FAWCETT

WITH TWO FIGURES IN THE TEXT

The first record of the infection of California citrus fruits by *Phomopsis* was made by Burger (1) in July 1919 on a shipment of lemons grown in Santa Barbara County. The seriousness of the citrus disease, known in Florida as stem-end rot, which, as shown previously by the writer (3), (2), is caused by the fungus *Phomopsis citri* Fawcett suggested further investigation.

In September 1919 the writer found a small number of fruits in packing houses in Santa Barbara County with a stem-end rot similar to that formerly described in Florida. From these fruits, a *Phomopsis* was isolated in culture. This decay, however, appeared to be of minor importance affecting very few fruits and only fruits that were quite mature when picked or that had been in the packing house for a long time. Since in the early stages this decay somewhat resembles other decays in lemon fruit due to *Botrytis cinerea*, *Sclerotinia libertiana* and *Diplodia* sp., it has probably been previously overlooked. The decay is usually lighter in color than the others mentioned, but can be distinguished only after some experience with it.

No fruit with stem-end rot was found in the orchards but after considerable search *Phomopsis* pycnidia were found on a few dead twigs, and suggestions of melanose (an important manifestation of *Phomopsis* attack in Florida) (5) were found on leaves. Some mature fruits picked from branches containing dead twigs and kept in the laboratory developed stem-end rot in about five weeks.

From a preliminary examination of cultures, the writer was inclined at first to think the fungus was *Phomopsis citri*. In April 1920, another search in Santa Barbara County revealed only a trace of the decay in one packing house. In the orchards after much search only a few pomelo fruits in one locality could be found with what appeared to be inconspicuous melanose markings. Later a *Phomopsis* similar to the Santa Barbara one was found by Dr. Bartholomew in some cultures taken from lemon fruits grown in Whittier, Los Angeles County. More recently the fungus has been found commonly by the writer in the bark

<sup>1</sup> Paper No. 99 University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.



of lemon trees affected with "shell bark," (4) and inoculation experiments have indicated that it may be a factor in the development of the shell bark disease.

#### INOCULATION EXPERIMENTS

Laboratory inoculation tests to compare the California *Phomopsis* with *Phomopsis citri* from Florida were made in January, 1920, at a temperature between about 15° and 20° C. On lemon fruits it took 32 days to produce the same effect with the California *Phomopsis* as was produced by the *P. citri* from Florida in 19 days. On immature pomelo fruits the California *Phomopsis* failed to produce rotting while the Florida fungus produced the decay in 19 days.

In another test started in May, 1921, the Florida *Phomopsis* initiated decay at 24° C. in mature lemon, orange, and pomelo fruits in 8 days and rotted entire fruits in 14 days, while under the same conditions the California *Phomopsis* failed to produce decay until after the lapse of two months.

In a later test (September 29, 1921) the California fungus failed in 18 days to produce decay in very mature Valencia oranges or on nearly mature lemons (two fruits each), at temperatures of 17, 20.5, 24.5, and 27° C. Inoculations were made by placing spores and mycelium on the stem end without puncturing. After the 18-day period all the fruits were left at about 20° C. It was not until 23 days later that *Phomopsis* rot was noted in one lemon each of the lots started at 17 and 27° C. Only after a third period of 45 days or 81 days from the beginning was *Phomopsis* decay noted in about half the oranges irrespective of the original temperatures at which they had started.

#### CULTURAL CHARACTERISTICS

The two fungi could readily be distinguished when grown on certain culture media. On glucose potato agar at about 20° C., in 20 days the California fungus showed a coarser growth with a tendency to form strands and fans with spaces between. The mycelium as a whole also tended to become light tawny with age and to turn to a cinnamon brown where it came in contact with this medium. The pycnidia were more abundant and tended to form only paraphyses and very few true spores. The mycelium of the Florida fungus, *Phomopsis citri*, on the other hand tended to remain pure white with a finer growth. The pycnidia was filled with true spores and contained few or no paraphyses.

On cornmeal agar the mycelium of the California fungus tended to turn to a warm buff while that from the Florida fungus remained pure white.

On sterilized orange twigs the California fungus produces numerous pycnidia with both true spores and paraphyses while *Phomopsis citri* tended to produce true spores only and no paraphyses under the same conditions.

On sterilized bean pods the pycnidia were more abundant than with *P. citri*. The true spores were abundant with both fungi, but the California fungus produced numerous paraphyses while *P. citri* showed none.

The growth of the two fungi was also compared at different temperatures, and some of the results are shown in table 1.

The growth of the California *Phomopsis* was found to have somewhat different temperature responses from that of the Florida fungus. (Tab. 1, and Figs. 1 and 2.)

TABLE 1

*Growth of Phomopsis californica and Phomopsis citri compared in terms of diameter of mycelium disks grown on soluble starch-glucose agar.*

MAINTAINED TEMPERATURE DEGREES C.	ORGANISM	NO. OF CULTURES	DAYS			
			2	5	8	11
			mm	mm	mm	mm
9	<i>P. californica</i>	2	0	2.0	2.9	6.6
	<i>P. citri</i>	2	0	2.5	3.7	8.6
14	<i>P. californica</i>	5	1.3	7.7	14.7	27.0
	<i>P. citri</i>	3	0	9.0	13.5	18.5
17	<i>P. californica</i>	5	4.1	16.2	30.3	39.9
	<i>P. citri</i>	3	4.5	13.3	22.1	31.1
20.5	<i>P. californica</i>	5	6.3	25.8	37.5	50.7
	<i>P. citri</i>	3	7.3	17.3	29.0	36.8
24	<i>P. californica</i>	5	5.1	18.2	30.2	41.2
	<i>P. citri</i>	3	8.2	22.0	31.6	42.7
27	<i>P. californica</i>	5	3.5	7.2	11.0	14.7
	<i>P. citri</i>	3	6.5	18.5	27.5	38.0
30.5	<i>P. californica</i>	5	2.1	4.7	7.6	10.0
	<i>P. citri</i>	3	7.0	13.5	20.5	26.7
34	<i>P. californica</i>	5	1.4	1.5	1.5	1.5
	<i>P. citri</i>	3	2.5	3.2	3.5	4.0
37	<i>P. californica</i>	1	0	0	0	0
	<i>P. citri</i>	1	0	0	0	0

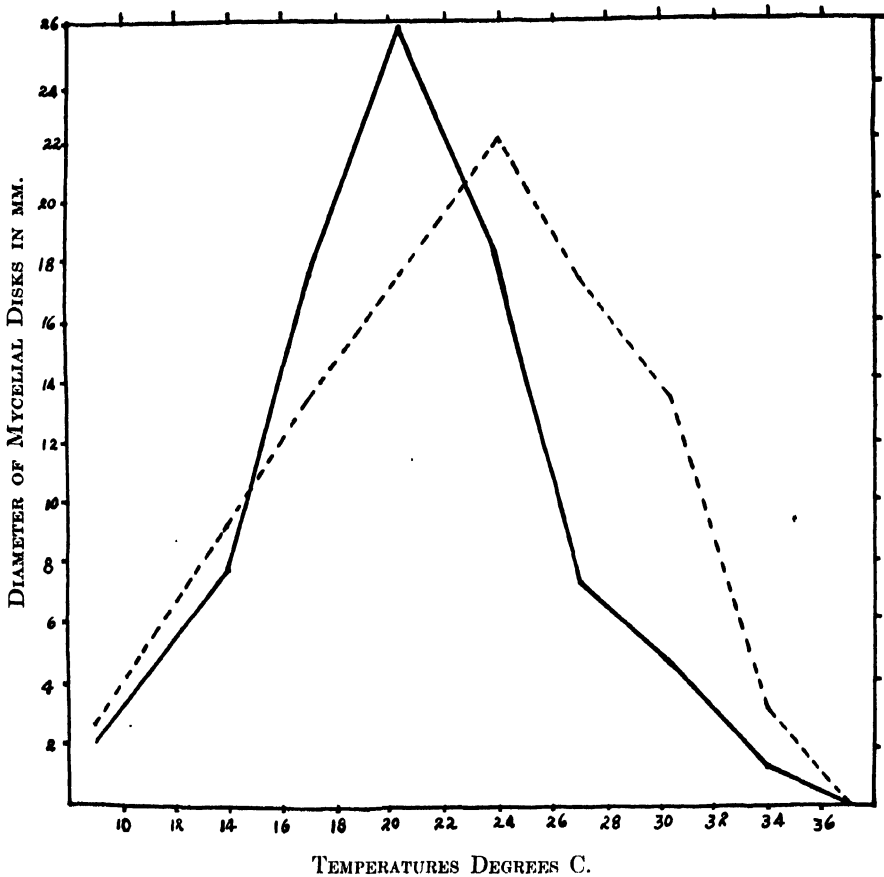


FIG. 1. Growth-temperature graphs for the first five day period on soluble starch medium (see table 1)

*Phomopsis californica* n. sp. —

*Phomopsis citri* Fawcett. - - -

On soluble-starch medium with one-half per cent sugar added, the California *Phomopsis* showed at the end of 4 days the greatest total growth at 20.5° C. while *Phomopsis citri* from Florida showed greatest growth at 24° C. The growth of the Florida fungus was also greater than the California *Phomopsis*, at all the temperatures tested above 24° C. The mycelial growth of the two fungi differed considerably, especially at higher temperatures. Both showed almost pure white growth initially at nearly all temperatures. The mycelium of the California fungus however, tended to become darker with age, especially at high temperature, and as a rule forms a more irregularly lobed margin. The California *Phomopsis* formed a thinner growth, less compact and less matted than that from Florida, at most of the lower temperatures.

The California fungus grown at 24.5° C. on soluble-starch agar for 10 days (Fig. 2, c) showed an irregular fern-like growth extending outward on the margins in the form of small separated fans with spaces between which tended to fill it later. *Phomopsis citri* (Fig. 2, e) with this same temperature and on the same medium showed a finer, more compact growth with more uniform distribution and more regular margins. At 27° C on this medium the California fungus (Fig. 2, d) grew very slowly as compared with *Phomopsis citri*; the latter grew quite well, forming a still finer more evenly distributed growth than at 24.5° C. (Fig. 2, f).

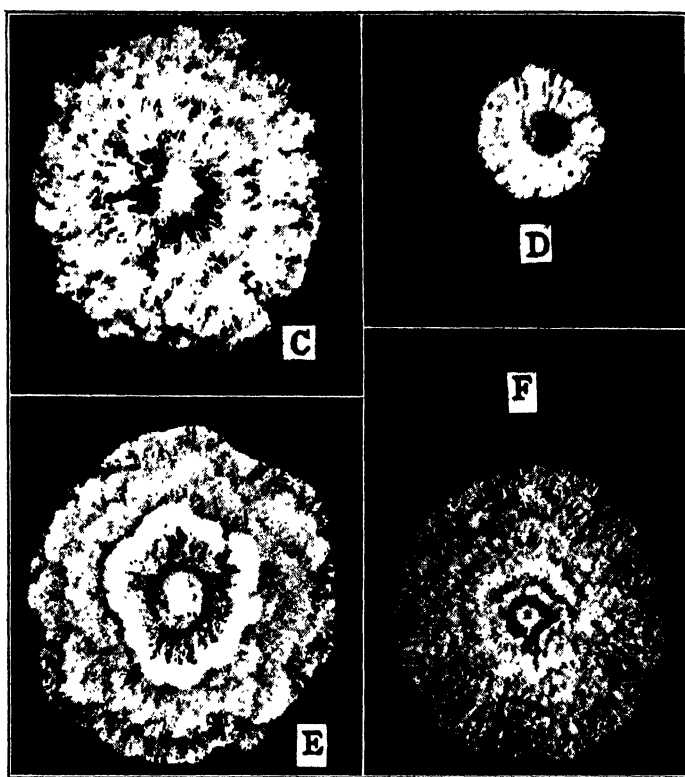


FIG. 2. (c) *Phomopsis californica* grown at 24.5° C. (d) Same fungus at 27° C. (e) *Phomopsis citri* grown at 24.5° C. (f) *P. citri* at 27° C. Natural size on soluble starch nutrient agar in 10 days.

The measurement of 100 spores of *P. californica* gave a mean length of  $8.4 \pm 0.7 \mu$  (range 7.7 to 9.1) and a mean breadth of  $3.9 \pm 0.5 \mu$  (range 3.4 to 4.3). A hundred paraphyses (sclerospores) gave a length of  $22.0 \pm 0.3 \mu$ , the range being 21.7 to 22.5  $\mu$ . The width was about 1.6  $\mu$ . These spore measurements are nearly like those of *Phomopsis citri* grown

under the same conditions, the measurements of 100 spores for the latter species giving  $8.4 \pm 0.6 \mu$  for the length (range 7.8 to 9.0) and  $3.8 \pm 0.5 \mu$  for the breadth (range 3.3 to 4.2). The two cannot therefore be separated on the measurements of the spores alone.

The weaker virulence of the California *Phomopsis* together with its lower optimum temperature for growth, difference in mycelial growth and difference in spore production (paraphyses rather than spores being the predominating characteristic on a variety of culture media), indicates that the fungus should probably be considered a different species from that commonly found in Florida. It is highly probable that the fungus occurred in California for a long time and has been previously overlooked because of its slight importance in producing decay. The following description may be added:

***Phomopsis californica*** n. sp. Pycnidia mostly clustered, sometimes rarely scattered, ovoid to conical, dark colored, 160–300  $\mu$  in diameter, erumpent. Ostiole 25–35  $\mu$  in diameter. Spores ovate, mostly rounded at both ends, sometimes nearly acute at one end, hyaline. Mean size of 100 spores measured,  $8.4 \pm 0.7 \mu$  long and  $3.9 \pm 0.5 \mu$  wide. Range 7.7–9.1 x 3.4–4.3  $\mu$ . Basidia about 15  $\mu$  long, paraphyses (sclerospores) 22–0.3 x 1.6  $\mu$ , curved at one end. Differs distinctly from *Phomopsis citri* Fawcett in coarser hyphae with tendency to form strands or fans of growth, the hyphae becoming cinnamon brown instead of pure white when in contact with glucose potato agar; also differs by forming principally paraphyses instead of spores on above media. On dead outer bark and in decaying fruits of *Citrus limonia* in California, United States of America.

Specimens of the fungus on lemon bark and on several different culture media are being sent to the herbarium of the Bureau of Plant Industry, Office of Pathological Collections, Washington, D. C.

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## OBSERVATIONS ON TWO POPLAR CANKERS IN ONTARIO

E. H. MOSS

### I. *Dothichiza populea* Sacc. and Briard.

Poplar canker caused by *Dothichiza populea* Sacc. and Briard is a well-established and destructive disease in Europe. In France, according to Delacroix (1), it is very troublesome to newly planted trees and in nurseries, attacking varieties of *Populus nigra*, but being particularly destructive to the Carolina poplar (*P. deltoides*). In America, the canker was reported for the first time in 1916 by Hedgecock and Hunt (2). These investigators have observed the disease in several of the New England and Northeastern States, where it is said to occur chiefly on *P. nigra* but also on *P. deltoides*. As far as the writer is aware no other paper on the subject has been published in America.

The writer's attention was first drawn to this disease in the spring of 1921 when he was asked by Dr. J. H. Faull to examine some young Lombardy poplars which had been planted in 1920 by the Harbor Commission of the City of Toronto, Canada. It was found that over 90 per cent of the trees—500 in number—were girdled by a fungus, which was identified as *Dothichiza populea*. In most cases the girdled area extended over a considerable length of stem and was located about two feet from the tip. From numerous pycnidia on the diseased parts spore horns appeared, the latter being in evidence as early as May 4.

Later in May, the disease was observed at widely separated points (in Middlesex and Oxford Counties) in southern Ontario, on old Lombardy poplars. In fact, almost every clump of these poplars examined showed evidence of the disease. In most cases, young shoots from the roots of the old trees were very markedly affected. These were being killed back, the young and immature leaves being blackened, and pustules appeared in large numbers on the stems. In most of the trees several branches situated at varying heights displayed evidences of the disease. Many of the older branches were disfigured by elongated open wounds, while younger shoots were being killed back.

Hedgecock and Hunt (2) observed only a few cases of infection of older trees by this pathogen. They suggest that the fungus has been

brought to America from Europe, but that it may have been brought in "previous to the enforcement of the present inspection laws." However, owing to the fact that *D. populea* has apparently not been collected or reported by American investigators, Hedgecock and Hunt come to the general conclusion that "it is a somewhat recent disease in the United States." Of course, the fungus may have been introduced on nursery stock on more than one occasion, but the writer's observations indicate that Hedgecock and Hunt are correct in their first suggestion. In Ontario the disease has undoubtedly been prevalent on poplars for many years, and consequently it is evident that *D. populea* is not recently new to America.

## II. *Cytospora chrysosperma* (Pers.) Fr.

The canker disease of poplar and other trees which is caused by *Cytospora chrysosperma* (Pers.) Fr. is apparently widely distributed in America. It has been reported as occurring in nine southwestern and central States by Long (4), in Idaho, Washington and Wyoming by Hubert (3), and in New York State by Povah (5). The present writer has observed the disease at various places in the neighborhood of Toronto and at Guelph, Ontario. The host most commonly attacked and most susceptible to the fungus in Ontario seems to be *Populus deltoides*. In addition, *Populus italica*, *P. balsamifera*, *P. alba* and *Acer saccharinum* have been observed as hosts. The latter is here reported as a new host for this pathogen.

According to Long, when *P. alba* is attacked by this fungus, the affected trees die branch by branch from the top downward. The writer has seen trees of *P. alba* and also Lombardy poplars (*P. italica*) which were being parasitized and slowly killed in this manner.

In a certain district in the vicinity of Toronto, it was observed that numerous young trees of *P. deltoides* were infected. Pycnidia of the fungus occurred near wounds in the younger branches and also on the lower parts of the trunks. As a result of these trunk infections some of the trees were being rapidly killed. It was apparent that the ground had been burned over and that the pathogen had attacked the trunks following fire injury. This observation is in accord with earlier accounts (3, 5) of this disease on poplars injured by fire.

## SUMMARY

Observations made during 1921 and 1922 make it clear that *Cytospora chrysosperma* and *Dothichiza populea*—two canker-producing wound parasites of the poplar—are not of uncommon occurrence in southern

Ontario. Furthermore, it would seem that *D. populea* has existed on this continent for a considerable length of time and that it has not been introduced from Europe as recently as has been suggested elsewhere.

Older trees of certain species of *Populus* commonly planted are rendered unsightly and are gradually killed by these fungi. Younger trees of *P. deltoides*, especially if seriously injured or weakened, are likely to succumb to the attacks of *C. chrysosperma*, and infected nursery stock of *P. italica* is rapidly killed by *D. populea*.

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# THE SPREAD OF TOMATO WILT BY INFECTED SEED

JOHN A. ELLIOTT AND R. F. CRAWFORD

WITH PLATE XXVIII AND TWO FIGURES IN THE TEXT

The first suggestion that tomato wilt is a seed borne disease was made by Clinton (1, 2) from the circumstantial evidence that the amount of wilt increased from year to year when seed from infected plots was saved for planting. No definite proof that seed carried the fungus was given by Clinton, but his suggestion has apparently been cited as established proof by Norton and Leathers (5). In his bulletin on the development of wilt resistant tomatoes, Pritchard (6) states that the disease is seed borne, but records no experiments establishing the fact and makes no citation of other work. His statement is as follows:

"Wilt is carried to some extent by the seed, but not so commonly as the high percentage of fruit infections in wilt-infested fields would seem to indicate. The fungus passes through the fibro-vascular bundles of the fruit to the seed and often invades the cells surrounding the seed coat. Were it not for the removal of these cells through fermentation and washing of the seed in the seed-saving process the infection of plants through the seed would be much more common. The fungus-bearing particles separated from the seed by fermentation frequently adhere to it, however, and thus become a source of infection for the plant and a means of more widespread distribution for the fungus. Infection of plants through the seed would be more common if tomato seed was produced commercially in badly wilt infested regions."

The nearest approach to proof that tomato wilt is seed borne, appearing in literature, is Edgerton and Moreland's (3) account, which might be said to establish the possibility that the disease can be carried on the seed. Edgerton and Moreland infected tomato seed with a pure culture of the wilt organism (*Fusarium lycopersici*) and succeeded in growing wilt infected plants from the infected seed the following planting season, proving that the fungus could live on the seed from the time the seed was collected to the planting time of the next year's crop in the region where they worked, a period of over three months.

Tomato wilt is the limiting factor in the successful production of tomatoes in Arkansas. In a large part of the state the disease is so generally present that no large plantings are undertaken, and even garden production of tomatoes for home use is attended by uncertainty

in most parts of the state. While so general a distribution of the disease might be expected in time from the introduction of the fungus on diseased plants, this explanation of its introduction into many fields, as has been pointed out by Edgerton and Moreland (3), is unsatisfactory. In Northwest Arkansas in particular, where the tomato canning industry has made a rapid growth and tomatoes are often grown from seed planted directly in the field, the general distribution of the disease in many such fields the first year they are used for tomato growing seems inexplicable on any other grounds than the introduction of the disease with the seed. This was so apparent in some virgin fields in 1920 and 1921 that very careful selection of seed from badly wilted plants in which the fungus had thoroughly invaded the fruit, was made in September and October, 1921, with the object of ascertaining whether such seed carried the wilt fungus.

The seed was cleaned by fermentation and washing, then dried and put away in an Erhlenmeyer flask plugged with cotton. The seed had a very clean bright appearance, but contained a considerably greater amount of small seeds than is usually found in commercial stock. The seed received no further attention until January 20, 1922, when planting was begun.

Since it was desired to isolate the wilt fungus only, the methods of isolation were designed to accomplish that end, and several modifications of the usual technique were tried. Very little plating was done by the poured plate method, but sterilized blotting paper was used instead. At first the seeds were placed, after various treatments, directly on the blotting paper; later, black paper was placed over the blotting paper and the seeds distributed over the surface of the black paper. Any fungous growth was very readily seen against the background of this black paper (Fig. 1). Fungi appearing on the seeds were examined microscopically and any which appeared to be the wilt fungus were saved for identification. The final test of identity as *F. lycopersici* was in all cases the production of typical wilt in tomato plants. However, the identity was established to a practical certainty by preliminary morphological and cultural tests. Any fungus which had the appearance of the wilt fungus when examined under the microscope was transferred to tubes of boiled rice, where, if it was *F. lycopersici*, it gave a very characteristic color reaction, which in our experience has always proved to be dependable (7). As nearly as it can be described from Ridgway's Color Standards and Nomenclature<sup>1</sup>, the color produced on rice was alizarine pink in

<sup>1</sup> Ridgway, Robert. Color Standards and Nomenclature. Published by the author. Washington, D. C., 1912.

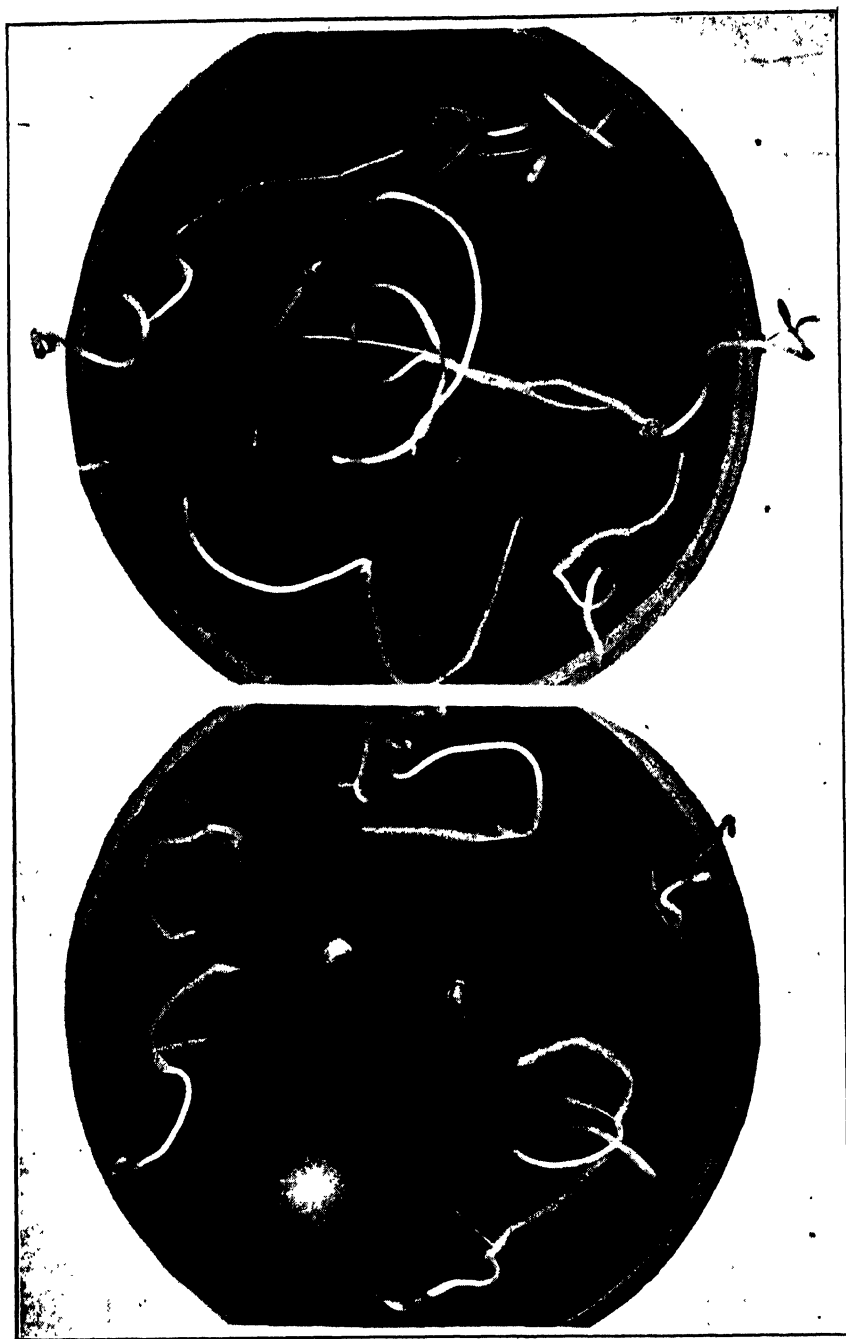


FIG. 1. Tomato seed from wilt infected tomato plants plated on sterilized black paper. Above, all seeds healthy. Below, 1 seed showing growth of *F. lycopersici*.

the lighter portions, and old rose, or Begonia rose, in the darker portions of the colony. The color perhaps approached a little nearer pure red than these, but no other colors given are so near it. All cultures giving this characteristic color reaction on rice produced wilt in tomato plants in subsequent tests.<sup>1</sup> Another modification in technique which was found useful in indicating the wilt fungus while growing on the blotting paper, was suggested by the color reaction given on rice. By dipping the white blotting paper in rice water before auto-claving the culture plates, the wilt fungus was made to produce a color reaction on the original plates, which aided in picking it out even when the growth of the wilt fungus was masked by some other fungus growing from the same seed (Pl. XXVIII). On the rice-starch-blotting-paper the fungus produced a deep red-violet color somewhat suggestive of the iodine starch reaction.

Three methods of treating the seed, previous to plating, were employed, as follows: 1. Seed was placed in sterile water in order to be easily separated, then transferred to the sterilized paper without any other treatment. 2. Seed was soaked for 2 minutes in 1-1000 mercuric chlorid solution, washed in sterile water and transferred to plates. 3. Seed was surface charred in concentrated sulphuric acid, washed in water, soaked for 2 minutes in 1-1000 mercuric chlorid solution and plated. Aside from *Penicillium*, which was evidently accidental contamination, and an occasional *Aspergillus*, the only fungus appearing on the plates,

TABLE 1  
*Fusarium lycopersici* isolated from tomato seed.

SERIES	DATE	TREATMENT	NO. SEED	ISOLATIONS	ISOLATIONS	FROM
				OF F. LYCOPERSICI	LIVING SEED	DEAD SEED
A	1/20/22	Soak in HgCl <sub>2</sub> . 2 min.	100	1	—	—
R	1/20/22	None	100	2	—	—
C	2/ 4/22	Soak in HgCl <sub>2</sub> . 2 min.	100	1	1	0
D	2/ 4/22	None	100	3	0	3
E	3/14/22	HgCl <sub>2</sub> 2 min.	100	0	—	—
F	4/17/22	None	100	6	0	6
G	5/ 4/22	Sulphuric acid, and HgCl <sub>2</sub> 2 min.	240	1	0	1
H	5/11/22	None	100	2	0	2
I	5/11/22	HgCl <sub>2</sub> 2 min.	100	0	—	—
J	5/11/22	Sulphuric acid, HgCl <sub>2</sub> 2 min.	150	3	0	3

<sup>1</sup> This same test was made in the case of the cotton wilt fungus, with which a practically identical color reaction was obtained.



FIG. 2. Tomato plants about three months old dying of wilt in pots inoculated with *F. lycopersici*. Right, pot inoculated with a known wilt culture. Pots at left inoculated with isolations A1, and B1. Other plants had died earlier.

aside from the wilt organism, was *Mucor sp.* which apparently came from the seed. This was true of all the seed plated, regardless of previous treatment. The most striking thing was that even the untreated seeds were practically sterile as far as fungus growth was concerned. The method of handling the seed gave little opportunity for the development of bacteria even if they were present. The results of the plantings are given in table 1.

The difference in the number of isolations of the wilt fungus from the surface sterilized and the untreated seed would indicate that the organism is carried on the outside of the seed coat, as a rule. More than 80 per cent of the seed germinated except when treated with sulphuric acid, when practically none germinated. At first no record was kept of the number of isolations made from dead or from viable seeds, but most of them came from dead seeds. The isolation of the fungus from the seed treated with sulphuric acid would indicate an internal infection, as it would seem impossible for the fungus to survive this treatment if it was not beneath the seed coat.

#### INFECTION EXPERIMENTS

The pathogenicity of the suspected fungi was tested by the method devised by Edgerton (4). Autoclaved pots of soil were inoculated with pure cultures of the fungi to be tested, by means of infected fragments of cotton stems, and the tomato seed planted at the same time. Check pots of known wilt fungus, and also uninoculated pots were run in the same series. Plants in the inoculated pots began to show infection in about a week and died of wilt from then on. Some of the plants lived for three months or more (Fig. 2). In the smaller plants the dark streaks due to infected vascular bundles could be seen without cutting the stems. No infections occurred in the uninoculated pots. Considerable

TABLE 2

*Inoculations with F. lycopersici isolated from tomato seed.*

ISOLATION SERIES	INOCULATED	1ST PLANT SHOWING WILT	
A 1.....	2/20/22	A 1	3/24/22
B 1, 2.....	2/20/22	B 1, 2	3/24/22
C 1.....	3/27/22	C 1	5/ 5/22
D 1, 2, 3.....	3/27/22	D 2, 3	6/ 1/22
F 1, 2, 3, 4, 5, 6.....	6/ 1/22	F 5	6/ 5/22
G 1.....	6/ 1/22	G 1	6/12/22
H 1, 2.....	6/ 1/22	H 1, 2	6/16/22
J 1, 3, 4.....	6/ 2/22	J 4	6/16/22
		D 1	6/5/22
		F 1, 2, 3, 4, 6	6/16/22
		J 1, 3	7/1/22

difference in the virulence of different isolations of the fungus was evident. In some pots the plants died very rapidly while in others they survived much longer. This difference in virulence of different cultures of *F. lycopersici* has been previously reported by Edgerton and Moreland (3). The results of the inoculation experiments are shown in table 2.

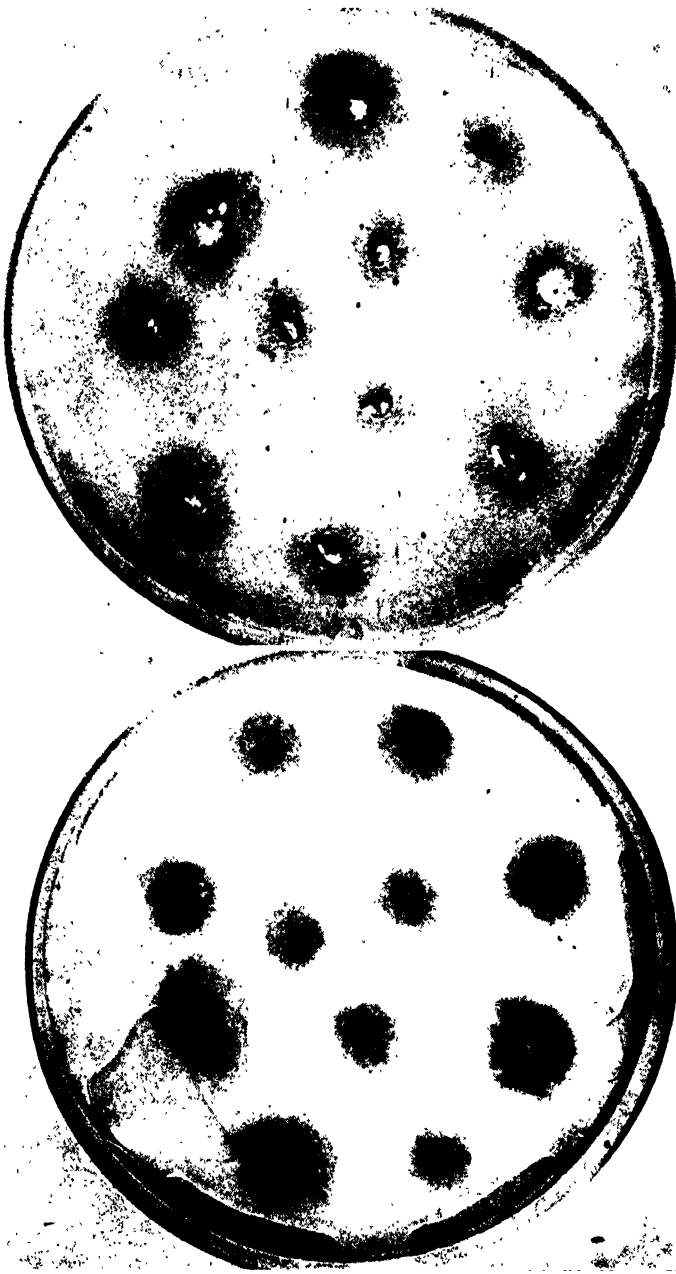
How long the fungus will remain viable on the tomato seed remains to be determined. These experiments show that naturally infected seed may carry the viable organism for at least seven months, a period long enough to carry the disease over from one season to the next in any tomato growing section of the United States. The seed was very carefully selected from fruits most heavily invaded by the wilt fungus but still the percentage of seeds from which the fungus was isolated was surprisingly high, being  $3\frac{1}{2}$  per cent in the case of the untreated seed, which should give the best index to the amount of wilt that seed from badly infected plants may be expected to carry.

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## TOMATO WILT

Tomato seeds artificially inoculated with spores of *F. lycopersici* plated on autoclaved rice-starch-paper, showing color reaction after 48 hours. Above, upper side of plate; below, lower side of same plate.





## A NEW SPECIES OF SCHIZONELLA

M. O. PARTHASARATHY IYENGAR AND M. J. NARASIMHAN

WITH FOUR FIGURES IN THE TEXT

This fungus was found growing on *Vitis quadrangularis* Wall. It causes a sort of witches' broom like growth at the nodes which at first sight might easily be mistaken for a kind of *Viscum* growing on the plant (Fig. 1). The diseased growth is very much unlike any part of the normal plant. These diseased parts start out at the nodes and branch repeatedly in all directions in a very irregular fashion so that the



FIG. 1. *Vitis quadrangularis* Wall. with the diseased growth on it caused by *Schizonella colemani*.

total growth at the node has more or less a spherical shape. These branches are pale green in colour with a reddish tinge towards the ends of the branches. A good number of dark green longitudinal swellings is found on these branches. Each of these green swellings when cut open shows a large quantity of dry, black, powdery spores lying loose inside. These green swellings are quite small near the ends of the

branches but become larger in size as the branches grow older and longer. The length of the swellings in the older branches is very nearly half an inch (Fig. 2).

SPECIES HITHERTO RECORDED (3)

1. *Schizonella subtrifida* on *Cirsium ochrocentrum*: spore 12–20  $\mu$  by 12–16  $\mu$ . American species.
2. *Schizonella melanogramma* on *Carex* spp.: spore 8–12  $\mu$  by 5–8  $\mu$ . American species.
3. *Schizonella melanogramma* var. *elynae* on *Elyna spicata*: spore 6  $\mu$ . Norwegian species.



FIG. 2. A few branches with one of the longitudinal swellings cut open showing the black spores inside.

DESCRIPTION OF THE MADRAS SPECIES

*Schizonella colemani*, n. sp.

Spores coupled in pairs through a narrow isthmus, each half being approximately semi-circular, the two together forming a sort of a dumb-bell (Figs. 3, 4). Epispore dark brown, smooth. Spore measurement 14–16  $\mu$  from end to end of the double spore by 7.9–10  $\mu$  across, being the average of ten measurements.

*Locality.* The specimen was collected once at Pallavaram and at another time at Vandalur, both stations very near Madras.

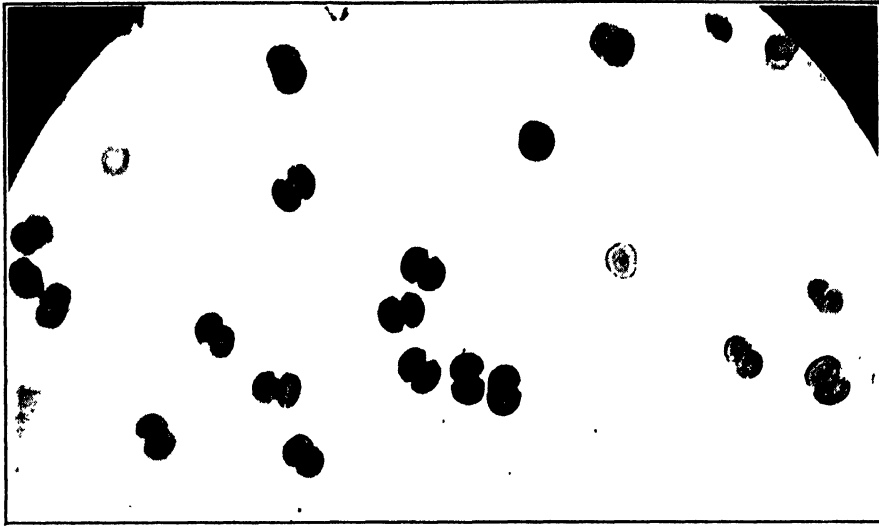


FIG. 3. Microphotograph of the double spores. C Zeiss Obj. 8 mm. OK. 12.

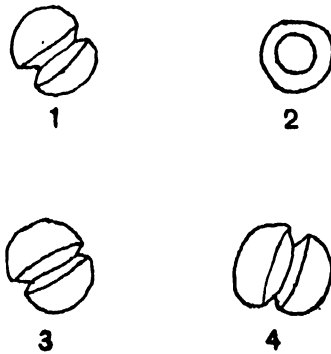


FIG. 4. Spores of *Schizonella*.  $\times 660$ .

#### GERMINATION

The spores did not germinate even though we tried hanging drop cultures in 1, sterilized water, 2, horse dung solution, and 3, malt extract. It may, however, be mentioned that the germination of the spores of the type species of the genus (*Schizonella melanogramma* D. C.) has been recorded. Rabenhorst (1) gives a figure of the germination of the spores of *S. melanogramma* and says "Das Promycel trägt die sporidien seitlich." Engler and Prantl (2 p. 12) with reference to the

spores of the same species say "Keimung wie bei *Ustilago*, mit hefeartiger Sprossung der Conidien." The cultural characters of the species under consideration will be studied later.

Our thanks are due to Dr. Leslie C. Coleman, Director of Agriculture in Mysore, for permitting us the use of his laboratory and library.

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### THE RELATION OF AIR TEMPERATURE TO THE MOSAIC DISEASE OF POTATOES AND OTHER PLANTS

JAMES JOHNSON

WITH ONE FIGURE IN THE TEXT

It has recently been shown by the writer that the optimum and maximum temperatures for the mosaic disease of tobacco are 28 to 30° C. and 36 to 37° C. respectively.<sup>1</sup> Similar experiments have since been conducted with mosaic diseases of various other plants, including potatoes, tomatoes, soy beans, pea beans, and clover, special attention being given to the potato mosaic. The method followed in potatoes has been essentially that of placing young potato plants showing symptoms of mosaic into the air control chambers, parts of the individual tubers in each series being represented in each chamber. Potato plants have been held at fairly constant temperatures for from one to three weeks at temperatures ranging between 6° and 36° C. The effect of temperature on the mosaic development was gauged by the intensity of the symptoms or the rate of "recovery" from the disease (Fig. 1).

The results have shown that the optimum and maximum temperatures for potato mosaic are considerably lower than those for tobacco mosaic. The most favorable temperature could not be determined closely since the disease is persistent at temperatures where the potato foliage makes little or no growth. Temperatures as low as 6° C. seemingly did not inhibit the disease. Taking the growth of the host into consideration, the optimum temperature lies between 14° and 18° C. Above 20° C. symptoms disappear, the rate of recovery from the dis-

<sup>1</sup> Johnson, James. The relation of air temperature to certain plant diseases. *Phytopath.* 11: 446-458, 2 fig., pl. 21 to 23. 1921.

ease being increased in proportion to increase of temperature within the limits of host development. To completely inhibit the disease, however, within a period of one to two weeks, a temperature of 24 to 25° C. is necessary; and this may be regarded as the maximum temperature for mosaic manifestation in the potato. New leaves, free from symptoms, appear quickly at this temperature and older leaves gradually lose their symptoms, the rate of "recovery" being roughly proportional to the age of the leaf, i. e., the older the leaf the longer the time required for recovery.

Potato plants and tubers infected with mosaic have been kept for varying lengths of time at 30° and 36° C. with the expectation that long treatment at such temperatures would eventually destroy the mosaic virus. Exposures as long as 10 days at 36° C. have failed to



FIG. 1. *The Influence of Air Temperature On The Mosaic Disease of Potatoes.* The foliage grown at a temperature of 25-26° C. lost all symptoms of the disease in seven days. Faint symptoms were still evident at 23-24° C.

entirely destroy the virus, although some indication has been obtained that longer treatment may be effective without destroying the germination of the tuber.

The response of the potato mosaic disease to temperature is in accordance with field observations on the behavior of the disease under different environmental conditions. It is important that such temperature responses be considered in experimental work with mosaic, and in testing tubers for seed, as well as in potato inspection work, and in certification records.

Certain other mosaic diseases have been worked with in less detail. These have all responded to temperatures in a manner similar to tobacco and potato, some falling in the high temperature class with tobacco, others behaving more like the potato mosaic, and others being intermediate in this respect. Mosaic of tomato (inoculated with the tobacco mosaic virus) is for instance, most active at high temperatures, whereas clover mosaic is seemingly favored by low temperatures. Soy bean mosaic is inhibited at temperatures of from 26 to 28° C., but the pea-bean mosaic can apparently persist at a considerably higher temperature.

WISCONSIN AGRICULTURAL EXPERIMENT STATION<sup>1</sup>

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## A STAINING METHOD FOR HYPHAE OF WOOD-INHABITING FUNGI

ERNEST E. HUBERT

In the course of the routine examination of various woods for the determination of decay, a rapid method for staining the hyphae of wood-inhabiting fungi has been developed. The methods developed by Diemer and Gerry (1) and by Sinnott and Bailey (2) give excellent results in differentiating the wood-rotting fungi from the host tissue but require from 12 to 24 hours for the staining process. Under ordinary conditions this time factor is not a serious drawback but where a large number of routine decay determinations are involved, a more rapid method is both necessary and desirable.

The following staining method is submitted to interested readers for trial. Any communications suggesting improvements or giving the results of trials will be appreciated.

After boiling the infected blocks ( $\frac{3}{8}$  inch cubes) in water for a half hour or more and soaking in glycerin-alcohol (50 parts glycerin and 50 parts 70 per cent alcohol), sections were cut and stained as follows:

1. Drain excess water from sections. Flood with bismark brown (2 per cent solution in 70 per cent alcohol) 1 to 2 minutes according to species of wood, density, thickness of section, stage of decay, etc.

2. Drain excess stain and wash with distilled water.

3. Flood sections for from 2 to 5 minutes with a solution of methyl violet made by mixing 4 parts of a saturated aqueous solution of methyl violet with 12 parts of distilled water. In some cases use full strength for from 1 to 2 minutes.

<sup>1</sup> Published by permission of the Director.

4. Drain excess stain and wash with distilled water.

5. Mount in water and examine for depth of staining. If violet color is faint repeat 3 and 4 using full strength stain for from  $\frac{1}{2}$  to 1 minute. If counterstain is faint, repeat, beginning at 1.

6. Dry slowly on warming plate using a cover glass to keep the sections flat. If sections curl use egg albumen or gum arabic fixative.

7. Add xylol and mount in balsam.

Hyphae of wood-destroying fungi, ascomycetes, molds, sap-stain fungi, etc., stain a deep violet color. The cell walls of wood stain yellow to brown. Wood tissues containing the cellulose-complex stain slightly with methyl violet giving a mixed brown and violet color. The wood tissues having the cellulose-complex mostly removed stain yellow to brown. The contents of the medullary rays and the bordered pits in conifers usually stain a violet color.

The drying method has been used in place of the ordinary method of dehydration by alcohol. The results secured seem to justify the means. The writer realizes that the drying method is contrary to the general practice but dehydrating with alcohol apparently removes the violet stain. Other methods of dehydration may be found practicable.

This method has been successfully used in staining and bringing into vision the minute hyaline hyphae of wood-rotting fungi which in the unstained sections are ordinarily invisible under the microscope. It has been tried out on a representative number of wood-inhabiting fungi and so far has given satisfactory results.

#### INVESTIGATIONS IN FOREST PATHOLOGY,

#### BUREAU OF PLANT INDUSTRY,

#### IN COOPERATION WITH THE

#### FOREST PRODUCTS LABORATORY, MADISON, WISCONSIN.

- (1) DIEMER, M. E. AND GERRY, ELOISE. Stains for the mycelium of molds and other fungi. *Science*, N. S. **54**: 629-630. Dec. 1921.
- (2) SINNOTT E. W. AND BAILEY, I. W. Some technical aids for the anatomical study of decaying wood. *Phytopath.* **4**: 403. Dec. 1914.



ABSTRACTS OF PAPERS PRESENTED AT THE SIXTH ANNUAL MEETING OF THE PACIFIC DIVISION, AMERICAN PHYTOPATHOLOGICAL SOCIETY, SALT LAKE CITY, UTAH, JUNE 22 TO 24, 1922.

*Fig smut studies.* ELIZABETH H. SMITH AND EDITH H. PHILLIPS.

In an investigation of the fig "smut" problem in California the following points were established by the writers during the fall of 1921. This refers to the infection of white varieties of fig fruit by *Aspergillus niger* v. Tiegh.

Time of infection. Infection was found to occur chiefly on the tree after the fruit is mature enough to be succulent but before much shriveling takes place. Infection may occur in firm, green figs. Development is most rapid just before the stage when picked for marketing fresh, and is entirely checked as soon as the pulp becomes brown and adhesive. This was proved by careful and extensive culture and inoculation experiments with figs of all stages on and under the tree.

Type of infection. The rot is firm, dirty white. It usually starts from the eye end and involves both skin and pulp in unshriveled figs, the surface remaining clean except in contacts. Pockets lined with the characteristic black spore masses are finally formed in the pulp.

Mode of infection. Mode of infection is almost entirely by the entrance of spores through the eye. Insects and smut spores were found in small hard figs, still sound, with closed eyes. Preliminary experiments indicate that insects, rather than wind, are the chief carriers.

*Notes on Synchytrium.* JAMES McMURPHY.

*S. papillatum* Farlow has recently been found on *Erodium moschatum* L' Her. A *Synchytrium* with spores 200 to 240  $\mu$  long in multicellular galls has been found on three species of *Lotus* (Hosackia).

*Morphological differences between Nectria galligena* Bres. and *N. coccinea* (ditissima). S. M. ZELLER.

*Nectria galligena* which has been described by Bresadola as the organism causing the European canker of apple and pear, is distinct from *Nectria coccinea* Fries (*N. ditissima* Tul.) to which until recently the cause of the canker has been attributed. These two species differ in their morphology as well as in their parasitic virulency and physiology. The perithecia differ in structure. In perithecia of *N. coccinea* found on various native hosts in Oregon the walls are made up entirely of pseudoparenchyma up to the ostiole, while in *N. galligena* from the same districts the pseudoparenchyma in the walls of the perithecia extends up about three-fourths the distance from the base. The remainder of the perithecial wall is of long, narrow cells, which radiate from the ostiole forming a cone. The perithecia of the two species are practically the same size and color. The ascospores of *N. galligena* are 14 to 22  $\mu$  in length, while those of *N. coccinea* are 9 to 14  $\mu$ . In Oregon collections the conidia (*Fusarium willkommii*) of *N. galligena* have the average measurement of 65.9 by 4 to 5  $\mu$  and those of *N. coccinea*, which are also a *Fusarium* spore, are 54 by 6  $\mu$ . The latter have more rounded ends and have a curvature of shorter radius than those of *N. galligena*. The conidial stromata of *N. coccinea* are orange colored, while those of *N. galligena* are creamy-white.

A "plum pocket" on *Prunus subcordata* in Oregon. S. M. ZELLER.

There occurs in Douglas and Jackson counties, infections of an *Exoascus* on the wild plum, *Prunus subcordata*, causing "plum pocket." This fungus differs from *E. pruni* and *E. communis*, its nearest affinities, in morphology and specialized host infection. It seemingly does not infect cultivated varieties of *Prunus*. A complete description of the species will be published soon.

Relation of rainfall to the late blight of *Phoma* rot of the sugar-beet. B. L. RICHARDS.

During the past season of 1921 a definite late blight of the sugar-beet became seriously epidemic throughout the northern part of Utah and southern Idaho. In Cache Valley, with a total acreage of 27,456 acres, 50 per cent of the fields were diseased. The disease in the field varied in severity from a fraction of 1 per cent of the beets destroyed to a total destruction of the crop. Many fields in the district were left unharvested, while others scarcely paid the expense of digging. The loss in the valley alone aggregated approximately two-thirds of a million dollars.

The available evidence indicates that the trouble is possibly identical with *Phoma* root-rot described by Edson and by various European workers. Data obtained from a study of this and similar but less severe epidemics of the disease in the Logan factory district during 1917 to 1919, show that such epidemics are favored by drought and, locally at least, are closely correlated with abnormally low precipitation during the months of June and July. This lack of rainfall, especially during June, appears definitely to create a critical and dangerous period in the life of the beet which our cultural methods and irrigation practices fail adequately to bridge over.

During certain years, as in 1921, the young beets owing to an abrupt cessation of the spring precipitation in May are forced into an early drought period during which they become so decreased in vitality that they fail to recover and with the advance of the season fall prey to soil organisms responsible for the late blight and root-rot of the crop.

Respiration of potatoes in relation to the occurrence of blackheart in storage. J. P. BENNETT AND E. T. BARTHOLOMEW.

Earlier work by Bartholomew and by Stewart and Mix, indicated that blackheart was due to a disturbance of respirational processes dependent on a temperature-time-oxygen relationship. Experiments with measured volumes of tubers and air in sealed chambers at different temperatures decreases with decreasing temperature from 40° C. to 50° C., then increases from 5° C. to 0° C. Decreasing oxygen concentration does not markedly affect the rate of respiration at temperatures below 35° C. until a very low concentration is reached.

The temperature-time-oxygen relation in the production of blackheart appears to be fairly definite. Between 40° C. and 5° C. decreasing temperature increased the period of exposure required to induce blackheart from eight to seventy-seven days; temperature below 5° C. decreased this period. At temperatures below 35° C. blackheart did not occur until practically complete exhaustion of the oxygen in the chamber; with increase of temperature above 30° C. an increasing amount of oxygen remained at the time of appearance of blackheart. Injury leading to the development of blackheart appears to be due to anaerobic processes in the tissues. At temperatures at which exhaustion of oxygen from the chamber precedes the appearance of blackheart, the injury may appear in any part of the tuber; at higher temperatures it usually occurs centrally.

*Potato blackleg with special reference to the etiological agent.* H. M. JENNISON.

Four different species of *Bacillus* have been described as the etiological agent of the blackleg disease of potatoes. In order to determine the relationships, some 12 strains of the blackleg pathogen, including the 4 "species" above mentioned, were studied comparatively as to morphology, cultural features and physiology. Special attention was given to the comparative study of the gas and acid producing function of the blackleg bacillus and this led to a quantitative study of carbohydrate utilization. An exhaustive examination and study of the literature was also made.

Below is presented a brief summary of some of the conclusions reached.

(1) The strains studied were morphologically similar. That they were specifically identical was abundantly proven by extensive comparative studies of their cultural features and physiology.

(2) The etiological agent of potato blackleg should be referred to as *Bacillus atrosepticus* van Hall.

(3) The following names are to be considered only as synonyms: *Bacillus phytophthorus* Appel., *B. solanisaprus* Harrison, *B. melanogenes* Pethybridge & Murphy..

(4) The Index Number 5312-32120-2110 is presented herewith in lieu of a fuller (revised) description of *Bacillus atrosepticus* van Hall.

(5) This pathogen develops acid and gas in the presence of a number of saccharides. The gas producing function is relatively weak, but this capacity can be built up to a certain extent by constant cultivation in the sugars which it is able to utilize.

(6) Quantitative determinations of carbohydrate utilization show that *Bacillus atrosepticus* cannot hydrolyze potato starch or dextrin.

(7) This organism secretes the enzymes invertase, lactase, maltase, as shown by its action on corresponding substrates.

*Corticium vagum as a factor in potato production.* B. L. RICHARDS.

In a series of pure culture experiments conducted under normal field conditions and in the greenhouse, several strains of *Corticium vagum* were found to produce severe and characteristic cankers on all underground parts of the potato. All strains, though able to infect mature stems either with or without wounding, were found to attack the plant most vigorously in its earlier stages of development. Growing points of the young shoots and of the stolons proved especially susceptible to attack.

In field experiments in which several hundred diseased hills were individually studied and carefully checked against disease-free hills, it was found that the fungus under conditions favorable for its pathogenic activity seriously reduced the numbers and size of the tubers, decreased the number of stems per hill, and greatly weakened the surviving vines. The latter were in general undersize, less vigorous, and died earlier than their disease-free neighbors. A comparison of the final yields from approximately five hundred diseased hills with an equal number of disease-free hills, grown in adjacent rows and under comparable conditions, showed that plants grown in cool soil infected with the Rhizoctonia stage of *Corticium vagum* on the average were reduced in yield to 50 per cent of that obtained from plants freed from the fungus by seed treatment.

Under natural conditions of potato culture, soil temperature proved to be the most important factor in determining loss to the crop.

*Hosts for Puccinia glumarum E. & H. in the United States.* CHAS. W. HUNGERFORD.

*Influence of the meteorological factors on potato disease and production in Colorado.* H. G. MACMILLAN.

Temperature and factors influencing temperature appear to affect the potato yield in Colorado. A critical study of meteorological factors and potato production indicates that temperature for at least six months prior to planting is reflected in the condition of the crop and the yield. Summer temperatures have much less effect. No data are available over many years as to the nature and causes of the prevailing disease, but *Fusarium* blight probably has been the controlling factor in potato production in Colorado. The high temperature in certain years, showing an increase above the normal during the winter and spring months appears to cause infection of the potato seed pieces or plants by the *Fusarium* fungus, with a rapid increase in the quantity of disease. An estimate of the winter and spring temperatures, showing whether they are above or below normal would be of great benefit to the farmer in preparing his seed potatoes. In years of high winter and spring temperatures he should plant whole seed. The critical temperature for infection of the potato seedpiece by *Fusarium* is about 14° C.

*Thielavia basicola* on watermelon in Oregon. M. B. MCKAY.

During the summer of 1916 diseased watermelon plants were received for diagnosis by the experiment station from Grand Island, Yamhill county, Oregon. The older leaves in the center of affected hills were dying prematurely, turning brown, wrinkling and drying up from the center of the hill outward, with the leaves on the outer and younger portions of the runners now about 18 inches long apparently unaffected. The grower suspected a leaf disease but examinations failed to disclose any evidence of such a malady. On the other hand portions of the outer tissues of the main stalk from one to four inches below the ground surface were from slightly to considerably disintegrated, giving them a scurfy appearance. Mounts from this tissue revealed the presence of a considerable number of chlamydo-spores which on culturing, gave typical chlamydo-spores and endoconidia of *Thielavia basicola* Zopf. Inoculations of young watermelon seedlings with this fungus in damp chambers have rather vigorous root rot in two week's time. So far as the writer is aware this fungus has not been found previously in Oregon on any plant nor does it appear to have been encountered frequently in the western states. O'Gara reported it as parasitic on watermelons in the Salt Lake Valley, Utah, in 1915.

*Distribution of Tylenchus dipsaci on wild strawberry in Oregon—Preliminary report.* M. B. MCKAY.

During 1921 the leaf and stem-infecting nematode *Tylenchus dipsaci* (Kühn) Bastian was found on wild strawberry plants in the vicinity of Siltcoos Lake in western Lane County. During the two previous seasons this pest had been encountered on cultivated strawberries and also on clover in the same general locality. Considerable interest was therefore attached to the occurrence of the disease on the wild plants, and a cooperative survey was arranged with several agencies participating to determine the approximate distribution of the disease in the coast regions of the state considered most apt to show the malady. To date the disease has been found on wild strawberry plants in four counties, namely Lincoln, Lane, Douglas, and Coos. In Lincoln county the diseased area found was very small, being only a few square yards in extent, and located a few yards from the ocean. In Lane county diseased plants were found scattered over an area about a mile in width and two miles long all within one and one-half miles of the beach. The diseased plants found in Douglas county are really an extension

of the infested area in Lane county and were found only a short distance over the county line. In Coos county diseased plants were found in an area extending back one-half mile from and along the beach for two and one-half miles. In this case the limits of the infestation have not been determined. The survey is being continued.

*Destructive rust (Puccinia subnitens Dietel) on spinach in the northwest.* H. P. BARSS.

On May 4, 1922, liberal samples of spinach leaves were forwarded to the Oregon Experiment Station by J. B. Wiley from the Walla Walla valley. These leaves were abundantly sprinkled with the bright orange cluster cups of a rust. The vegetable growers of the Walla Walla valley have sustained heavy losses on the spinach, both in the early and late crop. No losses from such a disease have been reported to the Oregon Experiment Station in previous years, and growers in the district report no commercial losses from this cause in previous years. Inasmuch as there appears to be no common rust disease accompanying spinach production in most parts of the world it was at once expected that some native rust had attacked the spinach. *Puccinia subnitens* Dietel was suspected and a search for the salt grass or alkali grass *Distichlis spicata* which is the telial host of this rust proved that the alkali grass was one of the three most common grasses growing in the vicinity of the spinach beds.

Specimens of this grass collected by Mr. Wiley close to the spinach beds were completely covered with the telial sori of *Puccinia subnitens*. Cultural experiments at Corvallis under experimental control have demonstrated the ability of the rust sporidia from this material to produce aecial infections on the cultivated spinach.

*Pathology of quaking aspen in Utah in relation to regulation.* E. P. MEINECKE.

Quaking aspen which covers large areas in Utah is at present without value as a timber tree. The growing scarcity of pulpwood points to the possibility of furniture utilization of quaking aspen, under the assumption that the species can be raised to merchantable size before decay destroys too much of the wood produced. The bulk of decay is caused by *Fomes ignarius*. Cull is low in younger age classes and increases with age particularly from the 91-100 age class on. Relative vigor of growth and character of wounding, besides age, influence the cull per cent. About 50 per cent of the wounds become infected. Wounds from fire commonly lead to infection and heavy cull. Elimination of fires and active control of injurious factors through elimination of infected trees are indispensable in the regulation of aspen stands. Since the probable silvicultural rotation of aspen will be shorter than the pathological rotation, it appears possible to raise quaking aspen for pulp in Utah.

*The toxicity of copper sulphate to the spores of Tillatia tritici (Bjerk.) Winter.* FRED N. BRIGGS.

*Stimulation by copper carbonate of wheat seedlings in the greenhouse.* FRED N. BRIGGS.

*Studies on Helminthosporium species found on cultivated barley in California.* G. E. PAXTON.

Studies carried on at Riverside, California, showed the mature perithecial stage of *Helminthosporium gramineum* to be present on two year old barley straw. On March 28, 1922, transfers were made of mature ascospores to cornmeal agar. Typical *Helminthosporium gramineum* conidia were produced. *Hordeum sativum* inoculated with the same gave typical lesions of this species.

Sixteen year old herbarium specimens of cultivated barley affected with *Helminthosporium gramineum*, when placed in a moist chamber, produced, after two weeks, conidiophores and conidia from the dormant mycelium. These conidia germinated readily in water and on potato agar. These conidia measured 100 to 110 by 12 to 15  $\mu$ .

The results of tests made with *Helminthosporium sativum* showed the optimum temperature for growth and sporulation to be about 30° C. Corn-meal agar proved to be a more favorable medium for growth than potato agar.

*Helminthosporium sativum* conidia from cultivated barley, *Hordeum sativum*, were grown in pure culture. Transfers from the conidia of this culture were made to squirrel-tail grass, *Hordeum muranum*, producing typical *Helminthosporium sativum* lesions and conidia. The reverse cross from squirrel-tail grass to cultivated barley gave like results.

*A Helminthosporium root rot of wheat in Idaho.* J. M. RAEDER.

A rather serious root rot of wheat was reported from Madison county, Idaho, last year. Investigations of the trouble disclosed the fact that it was confined to the dry land farms. The fields affected were spotted with diseased plants. These spots were irregular in shape and seemed to be confined to low swales or where snow had drifted the previous winter. The plants occurring on these spots were stunted and lighter in color. There was evidence that there had been considerable tillering earlier in the season, most of which had been killed. Counts made in the worst infected spots, showed only one in fifty plants remaining. Heads were formed on the remaining plants, but were very stunted and contained only shrivelled grain.

Closer examination of the affected plants, showed considerable discoloration of the sheath at the base. Occasionally a brownish, triangular shaped spot could be found on the lower first or second internode. Sometimes the lower nodes themselves were discolored.

Isolations were made from infected plants, which consistently gave a species of *Helminthosporium*. No work has been done to determine the species.

*A Fusarium blight of spinach.* CHAS. W. HUNGERFORD.

A rather serious spinach disease has recently been found in several localities in Idaho. The disease appears when the plants are quite small, causing a stunting of the plants, curling of the leaves and finally the death of the plants. A species of *Fusarium* has been constantly isolated from the interior of the crowns and roots of diseased plants. The characteristic symptoms of the disease have been produced upon plants grown in sterilized soil to which had been added a pure culture of the organism.

Dr. C. D. Sherbakoff, to whom a culture was sent for identification, has found that the *Fusarium* belongs to the *Marteilla-Elegans* section and is sufficiently different from all previously described *Fusaria* to be considered a new species.

*The effect of presprinkling with water upon the efficiency of certain potato seed treatments for the control of Rhizoctonia.* J. M. RAEDER and CHAS. W. HUNGERFORD.

Preliminary laboratory tests conducted at the Idaho Experiment Station have shown that the efficiency of both the corrosive sublimate and the hot formaldehyde treatments for seed potatoes is greatly increased by first sprinkling the potatoes with water and covering for either 24 or 48 hours. Cultures showed that all sclerotia were killed when potatoes were treated as follows: (1) Sprinkled and covered 24 hours then treated with formaldehyde 1 to 120 at 50° C. for 3 minutes. (2) Sprinkled and covered for 24 hours

then treated with formaldehyde 1 to 120 at 55° C. for 1 minute. (3) Sprinkled and covered 48 hours then treated with formaldehyde 1 to 120 at 50° C. for 2 minutes. Cultures were made from similar lots of potatoes which were not presprinkled before treatment with hot formaldehyde showed that control was not absolute.

Sprinkled with water and covering for 24 and 48 hours before treatment increased the efficiency of the mercuric chloride treatments somewhat but in no case was the control absolute. Field tests last year showed increased efficiency of sprinkling previous to treatment with mercuric chloride. Further field tests are being conducted this year.

*White pine blister rust in the Pacific Northwest.* J. S. BOYCE.

White pine blister rust (*Cronartium ribicola* Fischer) was found for the first time in the Pacific Northwest in the fall of 1921. The English black currant (*Ribes nigrum*) was found quite generally infected in the cultivated portion of western British Columbia. A few diseased eastern white and Himalayan pines were discovered in Vancouver.

In Washington, infected black currants were located at Sumas, Mt. Vernon, Everett, and Port Townsend. Two eastern white pines, killed by the parasite, were collected in a nursery at Mt. Vernon.

Scouting in the spring of 1922 has shown infection on native western white pine (*Pinus monticola* Dougl.) in western British Columbia to be general. As yet this spring no infected native pines have been discovered in Washington.

Observations show that the black currant is readily infected with a subsequent heavy spore production. This accords with the behavior of the same host in the east. Furthermore, western white pine seems to be very susceptible.

So far there is no indication of the disease in the commercial white pine region of Idaho or western British Columbia or in the sugar pine stands of southern Oregon and British Columbia. Every effort must be made to keep these regions free from infection.

*Symposium:* The relation of temperature and other factors to the etiology of plant diseases.

Discussion by Dr. C. W. Hungerford, University of Idaho, and Dr. H. S. Fawcett, Citrus Experiment Station. This symposium was held jointly with the Plant Physiologists.

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# PHYTOPATHOLOGY

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## SEPTORIA DISEASES OF CEREALS.<sup>1</sup>

GEORGE F. WEBER

WITH PLATES XXIX AND XXX AND FIVE FIGURES IN THE TEXT

### INTRODUCTION

It is the purpose of the present paper to bring together the results of investigations conducted during the past two years at the University of Wisconsin on the diseases of cereals and related plants caused by certain species of *Septoria*. The investigations have involved a study of these diseases in the field, greenhouse and laboratory as well as a study of herbarium material and a review of the literature. It has been found that this group of diseases is caused by closely related species of *Septoria*. However, by means of morphological and cultural studies of the organisms and inoculation experiments it has been possible to segregate these diseases very clearly.

The disease on oats has been found for the first time in the United States, and the ascigerous stage of the causal fungus has been developed in artificial cultures. This ascigerous stage has not as far as known been found under natural conditions. This fungus on oats has been found to be sharply specialized to the genus *Avena*. The common name "Speckled Blotch" of oats is here suggested for this disease on account of the speckled condition of the lesions caused by the presence of the scattered, black pycnidia. Accordingly the results of these investigations are given under the heading "The Speckled Blotch of Oats," caused by *Leptosphaeria*.

The investigations have verified previous work in showing that there are two diseases of wheat caused by two rather closely related species of *Septoria*. The results on these two diseases of wheat are given under the headings of "The Glume Blotch of Wheat" and "The Speckled Blotch of Wheat," respectively.

It has been found that the disease on rye, barley, quack grass (*Agropy-*

<sup>1</sup> This is the first of three articles by the author on *Septoria* diseases of cereals and some of the grasses.



*ron repens*), brome grass (*Bromus inermis*) and Kentucky blue grass (*Poa pratensis*) are each caused by distinct species of *Septoria*. In each case the fungus seems to be specialized to the genus or host on which it occurs. The results of these investigations are given in the order in which the hosts are mentioned above.

The seeds necessary for the propagation of the plants were obtained from the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.

The different species of *Septoria* were collected in the field in the vicinity of Madison, Wisconsin. During the past two years, observations have been made in the cereal plots on the Hill Farm of the University of Wisconsin, and in the plots of the Wisconsin Experiment Station at Madison, Wisconsin.

The inoculum used in the various experiments was obtained from pure culture and from diseased material collected in the field. The plants were inoculated by means of an atomizer, usually by spraying a spore suspension on the leaves of the host plants. In the field the inoculated plants were then covered with glassine bags for from 48 to 72 hours depending somewhat on weather conditions. The bags were then removed and the data were taken after about two weeks. In the greenhouse the inoculated plants were placed in a moist chamber for from 2 to 4 days and then placed on a greenhouse bench.

The writer takes pleasure in making acknowledgment to Dr. A. G. Johnson, for helpful suggestions, criticisms and guidance during the progress of the work, for aid in preparing this paper, and also for access to his collections of *Septoria* diseases on various cereal hosts collected at different points in the United States; also to Dr. J. J. Davis for herbarium material, literature references, and many helpful suggestions.

The writer also wishes to express his appreciation for the assistance rendered by Dr. Etienne Foex of Paris, in kindly lending at the request of Dr. A. G. Johnson, portions of type material from the original collections of J. B. H. J. Desmazieres.

## I. SPECKLED BLOTCH OF OATS CAUSED BY LEPTOSPHERIA

### INTRODUCTION

A disease of oats caused by *Septoria avenae* Frank, previously referred to by the writer (9), was found near Madison, Wisconsin, in September, 1921. The disease occurred on volunteer oats on the University farm. The plants were heavily attacked by crown rust and the scattered lesions

caused by *Septoria*, one to four on a leaf, occurred promiscuously intermixed with the rust sori (Fig. 1). The *Septoria* lesions were evident as rather small, circular to elongate, elliptical, killed and faded areas 2 to 4 by 2 to 8 mm. in size. The lesions were definitely distinguished from other spots on the leaf only by the presence of black, more or less scattered pycnidia. The development of the lesions as observed in inoculation experiments, discussed later, is briefly as follows:

The first visible symptoms after inoculation were observed on the eight or ninth day. Then spots in the leaf were readily detected. These spots were slightly lighter in color than the healthy parts. After 12 days the leaves were very mottled and the pycnidia could be distinguished. They were a light brownish color and very small. After 14 days the pycnidia turned black and most of the yellowed areas died forming spots light yellow to dirty white in the center, surrounded by a band of dull brown, and the whole surrounded by yellowish tissue blending into



Fig. 1. Portion of an oat leaf collected near Madison, Wisconsin, showing lesion caused by *Leptosphaeria avenaria*. Note black pycnidia in the lesion. The leaves also show a heavy attack of crown rust, *Puccinia coronata*. September, 1921.  $\times 26$ .

green. The central portion of the spot was dotted with scattered pycnidia. Infected oat plants were found only in two fields about half a mile apart; while other oat fields in the vicinity were carefully inspected, no evidence of the disease was found in them.

So far as the writer has been able to determine, the disease has not been previously reported in the United States. It has, however, been

found several times previously in other parts of the world. In 1847 Desmazieres (4) reported from France a *Septoria* disease on oats, and after examining it classified it as variety *C. avenae* of *Septoria graminum* Desm. In 1866 Moriere and Roberge (7) found also in France a *Septoria* disease on cultivated oats and listed it as *Septoria graminum* var. *C. avenae* Desm. Through the kindness of Dr. Etienne Foëx, Director of the Station de Pathologie Vegetale, Paris, portions of the type material of *Septoria graminum* var. *C. avenae* have been examined and found to be distinct from *Septoria avenae* Frank. The pycnosporos as described are identical with *Septoria graminum* Desm. Hence the pycnosporos of var. *C. avenae* Desm. are longer and distinctly narrower than *Septoria avenae* Frank. In 1892 Cobb (3) reported a *Septoria* disease on oats occurring in Prymble, England. He gave no description of the disease or organism nor did he mention the species of oats attacked. In 1895 this disease was reported on oats in Pomerania, Germany by Frank (6). He gave a complete description of the organism and named it *Septoria avenae* n. sp. He found the disease on the lower leaves of *Avena sativa*. The disease and fungus found by the writer agree in every respect with the description given by Frank. Thus the disease has now been reported from England, Germany, and the United States (Wisconsin). In nature it has been found only on *Avena sativa*. From inoculation experiments, reported later in this paper, it has been found that *Avena barbata*, *A. brevis*, *A. nuda*, *A. strigosa*, and *A. fatua* are also susceptible. Hence in the United States, up to the present time, this disease has been found developing naturally only to a limited extent on *Avena sativa*, and thus far it seems to be of negligible economic importance.

#### TAXONOMY AND MORPHOLOGY OF THE FUNGUS

In 1895 Frank (6) described a *Septoria* sp. on *Avena sativa* from Germany and named it *Septoria avenae* n. sp. The writer in September, 1921, found a *Septoria* sp. on *Avena sativa* near Madison, Wisconsin, which agreed with the description of Frank. The fungus was isolated by making single spore isolations and grown on potato-dextrose-agar and oat-meal agar. A number of transfers were made to each of these kinds of agar. In January, 1922, perithecia, with mature asci, were found both in the oatmeal agar cultures and in the potato agar cultures. In each case, as previously stated, cultures were made by isolating single pycnosporos. They were obtained from pycnidia from oat leaves collected in the field. To date (April, 1922), perithecia have been found in at least fifty different culture tubes. Mature pycnidia were also found in practically all of the cultures. The ascospores germinated in

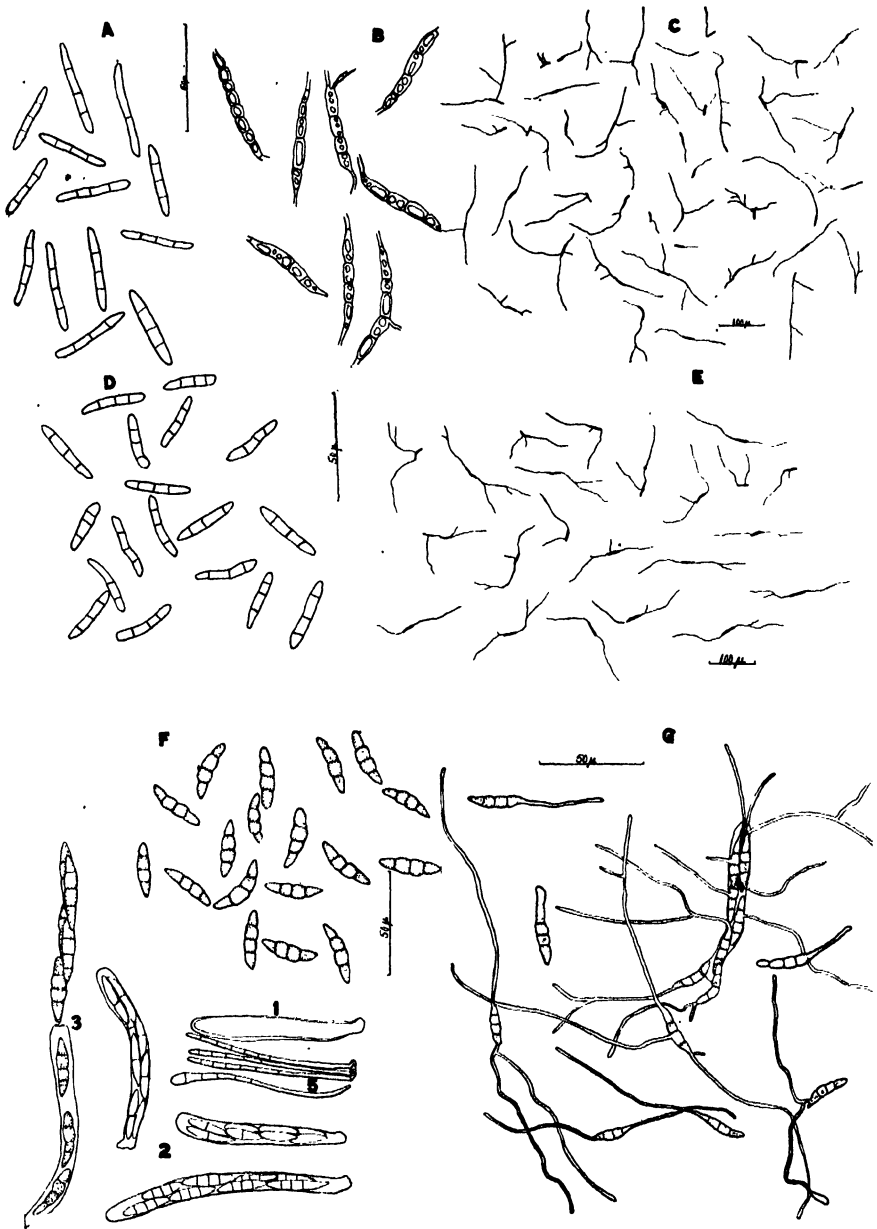


Fig. 2. A. Pycnospores developed on oat leaves in the field. B. Vacuolate condition of pycnospores 36 hours after germination. C. Germination of pycnospores after 24 hours, collected on oat leaves. D. Pycnospores grown on potato-dextrose agar. E. Germination of pycnospores after 24 hours, grown on potato-dextrose agar. F. Asci: (1) empty, (2) immature, (3) mature, (4) ascospores and (5) paraphyses of *Leptosphaeria avenaria*. G. Ascospores germinating in water after 18 hours. (Camera lucida drawings).

water in about four hours and on potato agar in seven hours. Ascospores in a water suspension were floated out on the surface of potato agar, poured plates and incubated twelve hours. Single spores were then transferred to culture tubes. After three weeks typical pycnidia began to appear in these cultures. About three weeks later perithecia began to develop and soon were numerous. Using ascospores developed in culture, inoculations were made in the greenhouse on seedlings of *Avena sativa*. Typical *Septoria* infections resulted in about two weeks. These infections were identical with other infections produced by inoculating oat seedlings of the same age with pycnospores obtained from pycnidia from oat leaves collected in the field. In both series the reisolated pycnospores from the inoculations made with ascospores and those made with pycnospores could not be distinguished. Pycnospores collected in nature were cultured and compared to cultures made from pycnospores developed from ascospores and those made with pycnospores could not be distinguished. Pycnospores collected in nature were cultured and compared to cultures made from pycnospores, developed from ascospores. The two strains of pycnospores, germinated in the same manner, the hyphae were septate, of the same size and branched in the same manner. The mycelium in general was of the same color and growth in the culture was of the same sort in each case.

From this evidence it is clear that the ascigerous stage of *Septoria avenae* has been found. From its characteristics, described later, it clearly belongs in the genus *Leptosphaeria*. It seems to be distinctly different from any described species of this genus. The fungus described by Auerswald (1) in 1869, on oats as *Leptosphaeria avenae* is distinct in that the ascospores of that fungus are considerably smaller, measuring 3.5 to 4 by 15 to 16  $\mu$  and are hyaline, in contrast to the larger, colored ascospores of the form found to be the ascigerous stage of *Septoria avenae* Frank. Therefore the following new combination is proposed and a description of the stages follows:

***Leptosphaeria avenaria* sp. nov.**

*Septoria avenae* Frank

Berichte der Deut. Botan. Gesell. 13: 61-65. 1895.

Not *Septoria graminum* var. *C. avenae*

Ann. Des. Sci. Nat. 3rd Serie 8: 9-18. 1847.

PYCNIDIA

Pycnidia are more or less scattered, often in rows, sub-epidermal, globose to sub-globose, visible to the unaided eye, 90-150  $\mu$  in diameter,

averaging about 120  $\mu$ . Wall is smooth, brown to black in color, composed of one to three layers of pseudoparenchymatous cells. Ostiole, round to oval, slightly elevated, 20–30  $\mu$  in diameter.

#### PYCNOSPORES

Pycnospores (Fig. 2, A and D) are rod shaped, straight or slightly curved, cylindrical, with rounded ends, three septate when mature, hyaline, guttulate, usually one or more guttulae on each side of the septa, 3–4  $\times$  25–45  $\mu$  averaging 3.5  $\times$  38  $\mu$ .

#### PERITHECIA

Perithecia were grown in test tubes at room temperature from pycnospores on potato-dextrose-agar, slightly acid. They were slightly embedded, globose to sub-globose, 60–130  $\mu$  in diameter. Walls smooth, black pseudoparenchymatous, thin, 2–3 cells thick. Ostiole usually round, not protruding, 12–20  $\mu$  in diameter.

#### ASCI

Asci (Fig. 2, F) are narrowly clavate with rounded tips, hyaline, thin walled, 10–18  $\times$  30–100  $\mu$ , averaging 15–50  $\mu$ . Each ascus contained eight spores biserially arranged.

#### ASCOSPORES

Ascospores (Pl. XXIX B and C) are fusoid, straight or slightly curved, ends obtuse, rounded, three septate, constricted at the septa, especially the center septum, second cell from top usually swollen, light yellow to slightly olivaceous, 4.5–6  $\times$  23–28  $\mu$ , averaging 5  $\times$  25  $\mu$ .

#### PARAPHYSES

Paraphyses (Fig. 2, F) are narrowly cylindrical, hyaline, septate, with slightly enlarged, rounded tips, 2  $\times$  60  $\mu$ , terminal cell 2–4  $\mu$  in diameter.

#### MYCELIUM

Mycelium grown in culture from either pycnospores or ascospores is hyaline, much branched, septate, with hyphae about 2  $\mu$  in diameter. The contents of the younger hyphae are either homogeneous or very minutely guttulate especially near the septa. The older hyphae become distinctly vacuolate. The mycelial colonies grown on agar are white with a very slight pinkish tinge.

## PHYSIOLOGICAL STUDIES

## Cultural Studies

The fungus grows readily in artificial culture. Numerous media were used and the mycelium developed well on all of them except string-bean agar in which case the surface seldom was completely covered and the growth was usually scant. Characteristic growth was produced on sterilized corn meal, wheat heads and stem of *Melolitus alba*. The most favorable media proved to be potato-dextrose agar and oatmeal agar. The growth on these two media was especially vigorous and rapid. Pycnidia began to form at the end of about four weeks and at the end of about eight weeks mature perithecia were found on both of these media. The pycnospores and ascospores developed in the pycnidia and perithecia respectively, germinated readily. The mycelium developed by the pycnospores grew on potato-dextrose agar very much the same as the above description. The mycelium from the ascospores on potato-dextrose agar developed rapidly and was of a slightly pinkish white color when several days old. After about two weeks pycnidia began to form along the upper edge of the slant where the medium was thin (Pl. XXIX, A). The formation of the pycnidia was accompanied by a change of color of the mycelium from white to grayish. After four weeks perithecia were also found in the same tubes in which the pycnidia were found. Both forms were mature and compared exactly with the pycnospores germinated after 3-4 hours in water in the manner described later.

## Germination Studies

*Pycnospores.* Pycnospores (Fig. 2, B, C and E) taken from the pycnidia on oat leaves collected in the field as well as those grown in pure culture on culture media showed indications of developing germination tubes after three hours at room temperature, when placed in a drop of water on a glass slide. When placed on the surface of potato-dextrose agar no evidence of germination was observed until after eight hours. The pycnospores usually produced germ tubes from each end and occasionally from the sides. Four germ tubes have been observed developing from a single pycnospore in water. The most favorable temperature for spore germination was between 20-24° C. At this temperature the spores germinated in the shortest time and the growth was most rapid and vigorous. At the lower temperatures the time before germination was lengthened, although germination took place at 4° C. after 48 hours. At the higher temperatures the time for germination was shorter but the

germ tubes did not appear to be as turgid. No germination took place above 36° C.

*Ascospores.* Ascospores (Fig. 2, G) developed on potato-dextrose agar and oatmeal agar were removed from the perithecia and placed in distilled water on glass slides or placed on clarified potato agar. The spores in distilled water showed the formation of germ tubes after four hours at room temperature. On the potato media the first germ tubes were not observed until after seven hours. The germ tubes were developed first at the ends of the spore and soon after one of the remaining two

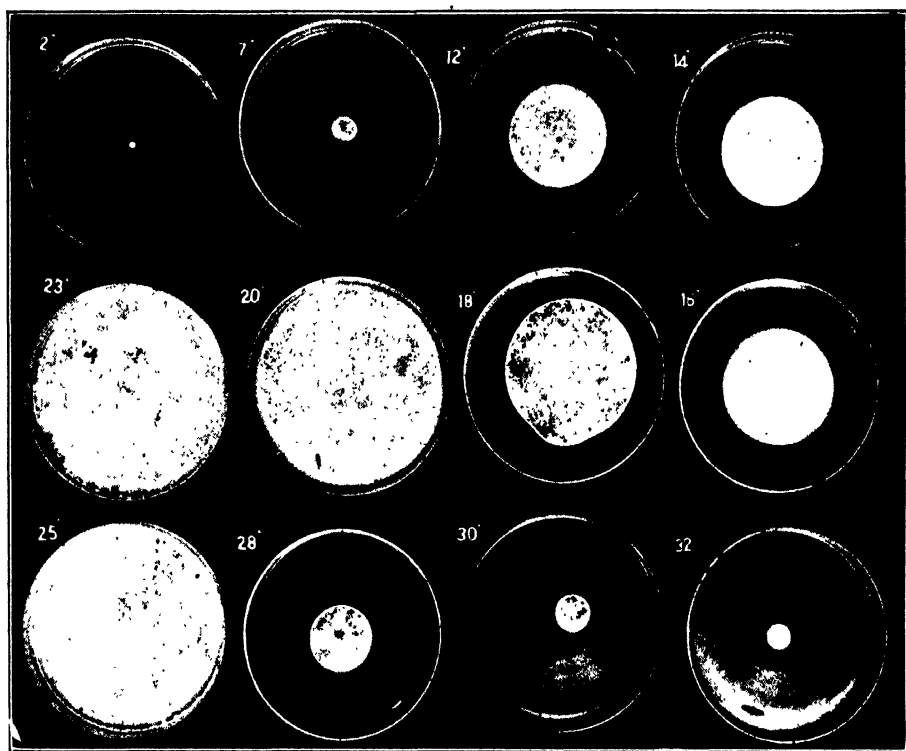


Fig. 3. *Leptosphaeria avenaria* grown 12 days on potato-dextrose-agar at various centigrade temperatures as indicated, showing extent and type of growth of each. One fourth natural size.

cells developed a germ tube. The germ tubes were hyaline, rounded at the growing tip, septate, and immediately sent out branches forming a mycelial mat. Ascospores retained within the ascus germinated (Fig. 2, G) as soon as those that were free in the water. The germ tubes from these ascospores broke through the ascus wall at many different places



along the sides as well as growing from the openings at the tip and base of the ascus. It was also observed that ascospores which were accidentally broken into two parts germinated from the end cells in the same time as uninjured spores. When germinating in a drop of water on a glass slide the ascospores and germination tubes adhered very persistently to the glass surface, remaining in position in running water.

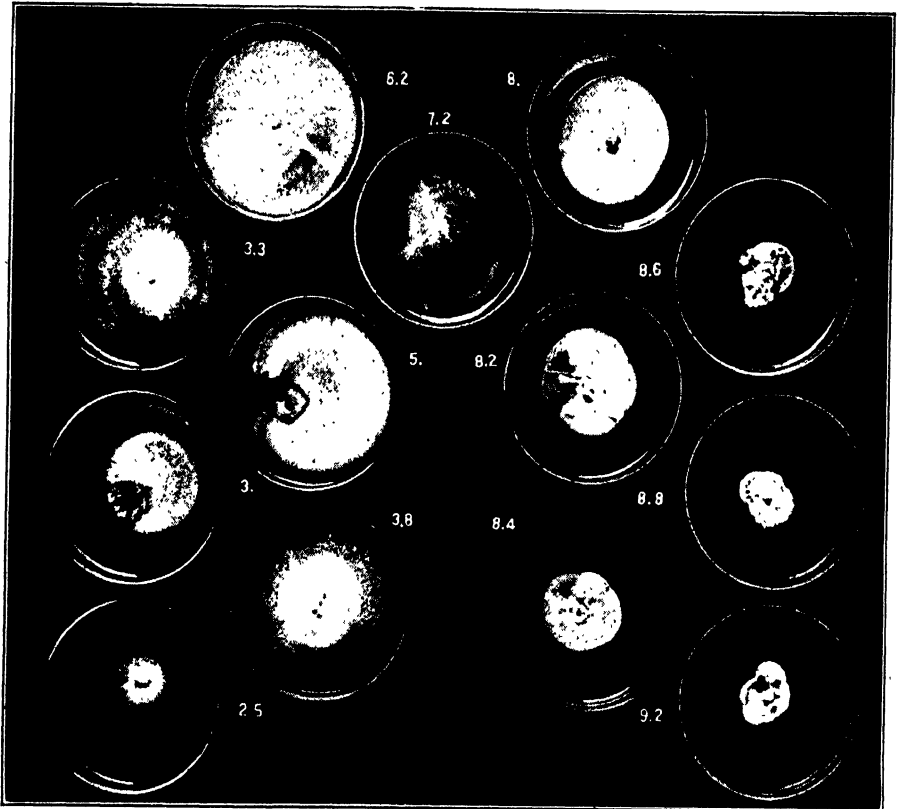


Fig. 4. Organism grown on acid and alkaline potato-dextrose-agar adjusted to the pH values as indicated, showing extent and type of growth to each reaction. One-fifth natural size.

The superfluous water was removed from a group of ascospores which had germinated twelve hours previously and diluted India ink as used by Errera (5) and Boyle (2) was placed on the ascospores and then covered with a cover slip. Examination with the microscope showed that a light colored area surrounded every germinated ascospore and also the older portions of the germination tubes. At the growing tip

there was little or no evidence of this gelatinous sheath. It gradually increased in extent toward the spore (Pl. XXIX, B). No doubt, as pointed out by Boyle (2) gelatinous sheaths of this sort may play important rôles in initial infections in making it possible for the spores and germination tubes to adhere tightly to the cuticle of the host.

#### The Relation of Temperature to the Growth of the Organism in Culture

The relation of temperature to the growth of the fungus was studied by growing the mycelium on poured plates of potato-dextrose agar incubated at different temperatures (Fig. 3). Bits of mycelium were transferred to the center of each plate from a common source and the plates were placed in incubators and left there until the mycelium at the most favorable temperature had covered the surface of the plate. It was found that little or no growth took place at 2° C.; the most abundant growth was at 20–25° C. From 25° C. upward growth decreased until at 32° C. where very little growth took place. Hence the cardinal temperatures for mycelial growth would be approximately as follows: minimum 2° C., optimum 23° C., maximum 32° C. There were decided decreases in growth below 12° C. and above 25° C.

#### The Relation of the Reaction of the Medium to the Growth of the Organism in Culture.

The fungus was grown on potato-dextrose agar to which had been added different amounts of  $\frac{1}{4}$  HCl or  $\frac{1}{4}$  NaOH. The agar was prepared and made neutral to phenolphthalein, then 10 cc. of the medium were placed in each test tube to which was added one or more drops of  $\frac{1}{4}$  HCl or  $\frac{1}{4}$  NaOH. Thirteen tubes made up a series which was found to range from pH 2.5 to pH 9.2 (Tab. I). Ten series were made up in the same way. Five series were used as controls, two were tested for acidity by the Fuller scale and the other three by Clark and Lœb's colormetric method for determining H-ion concentrations. The five remaining series were poured into petri dishes and inoculated with the fungus from a single source, and placed at room temperature in the dark for growth. The experiment was concluded when the fungus covered the surface of any petri dish. After fourteen days the pH 6.2 petri dish cultures were covered by the fungus. The growth was decidedly less at pH 7.2 and gradually decreased up to pH 9.2 where very little growth had taken place. From pH 8.6 to pH 9.2 the growth of the fungus was not characteristically normal. The margins were irregular, some dark spots appeared and the surface of the colony was very irregular when

compared with the colonies in other petri dishes of the series (Fig. 4).

It shows that the fungus grows best on a medium that is slightly acid. At H-ion concentrations lower than pH 3.8 and higher than pH 7.0 the growth was retarded.

TABLE I.

*Summary showing the amount of neutral potato agar used per tube, the number of drops of  $\frac{n}{1}$  HCl or  $\frac{n}{1}$  NaOH added to each and the reaction of each four days after preparation both in Fuller's scale readings and pH values.*

Tube Number	Agar cc.	Drops of $\frac{n}{1}$ HCl	Drops of $\frac{n}{1}$ NaOH	Dist. water in cc. added	Fuller's scale reading	pH values
1	10	6		5	+ 40	2.5
2	10	5		5	+ 30	3.0
3	10	4		5	+ 25	3.3
4	10	3		5	+ 20	3.8
5	10	2		5	+ 15	5.0
6	10	1		5	+ 10	6.2
7	10	0	0	5	+ 0.2	7.2
8	10		1	5	+ 0.3	8.0
9	10		2	5	+ 0.2	8.2
10	10		3	5	+ 0.3	8.4
11	10		4	5	+ 0.4	8.6
12	10		5	5	+ 0.4	8.8
13	10		6	5	+ 0.5	9.2

## PATHOGENICITY

## Isolation, Inoculation, and Reisolation

In inoculation experiments both in the field and in the greenhouse the fungus has been found repeatedly to be pathogenic on the leaf blades and leaf sheaths of oats. In inoculation experiments infection has not been obtained on the culm, panicle or mesocotyl.

The fungus was isolated from diseased areas on oat leaves by obtaining the pycnospores from the pycnidia, by placing the diseased area in distilled water for about fifteen minutes.

The surfaces of agar poured plates were inoculated with the spore suspension. After twelve hours germinating spores were observed with the aid of a microscope and marked for transfer. Single germinating spores were then transferred to slanted agar tubes. The surface of the slants were covered by the fungus in six to eight days. After six or seven weeks pycnidia and perithecia were formed in the test tubes. Mature pycnospores were obtained from the cultures and used suspended in

water as inoculum. Oat seedlings were inoculated with an atomizer and placed in a moist chamber for four days. These seedlings were dried every day and sprayed with distilled water and covered every

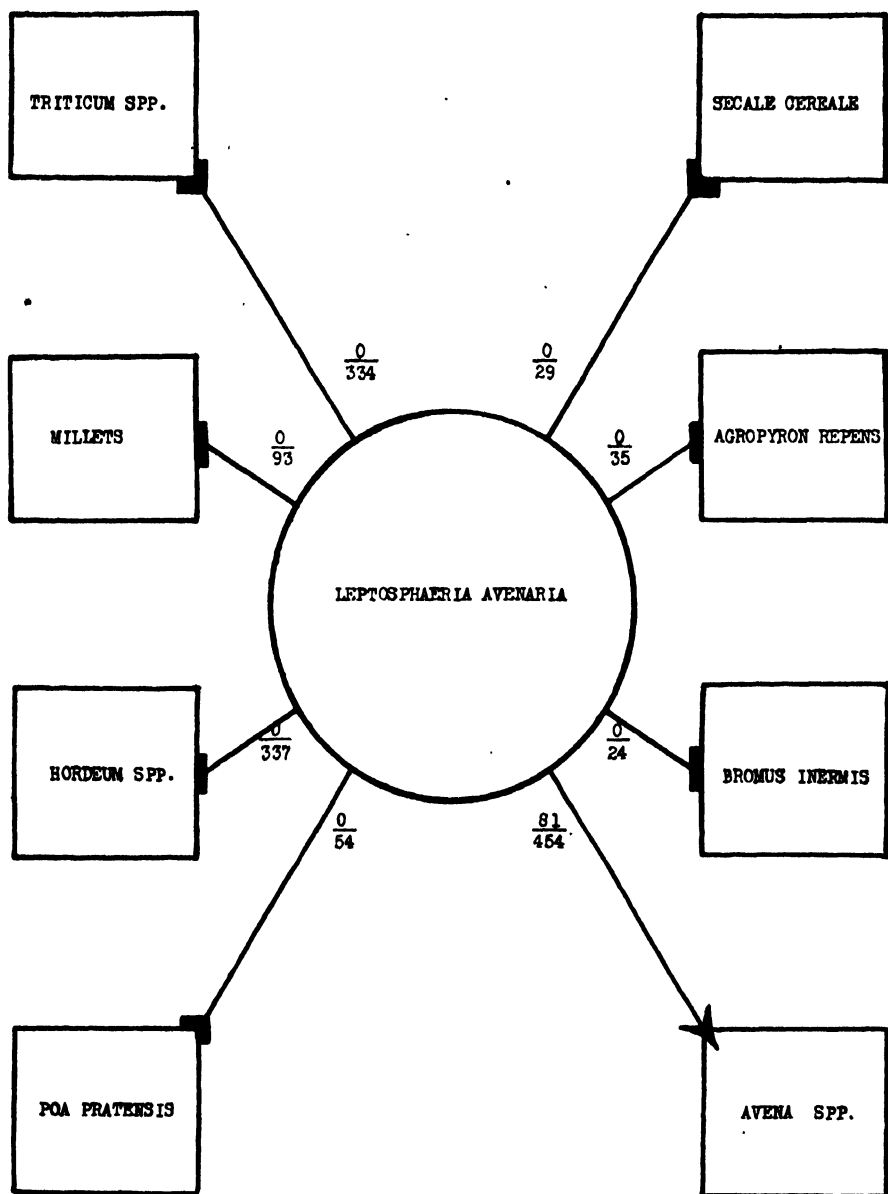


Fig. 5. Diagram summarizing results given in table 2. Inoculum from center circle, hosts inoculated in rectangles. Denominator of fraction designates number of leaves inoculated and numerator signifies the number which became infected.

night during the four days; they were then placed on a greenhouse bench. After fifteen days characteristic pycnidia were developed on the inoculated leaves while the uninoculated plants remained healthy. A parallel series of inoculations were made in which the inoculum was obtained directly from oat leaves collected in the field. Two weeks after inoculation pycnidia developed which were identical with those developed on the plants inoculated with pycnosporos grown in culture. Reisolations were made from both series and the organism obtained, in the form of pycnosporos, mycelium and pycnidia was identical with the organism from the original material. Inoculation experiments were also conducted in which the inoculum consisted of water suspensions of ascospores developed in culture. The inoculation methods were the same as previously described for inoculations with pycnosporos. About two weeks after inoculation, infections were obtained on the leaves of oat seedlings. All species listed in table 2 were found susceptible.

#### Cross Inoculation Experiments

The disease was found in the field only on *Avena sativa*. Experiments have been conducted in an effort to determine the host range among the species of *Avena*, the other cereal crops and certain related grasses. In no case has infection been obtained on any host other than certain species of *Avena*. Among these species there is considerable variation as to susceptibility to the disease. The reproduction of the disease has been somewhat erratic. In certain experiments some species of *Avena* were susceptible and in other experiments identical results were not always obtained even when the same methods were used. Disturbed greenhouse conditions may have been responsible for some of the discrepancies. The results of the inoculation experiments are given in table 2 and figure 5.

From the results given in table 2 it will be noted that infections were obtained only on species of *Avena*, indicating distinct specialization to this genus. Certain species seem to be difficult to infect, especially *Avena fatua* and *A. strigosa*.

TABLE 2.

Summary of results from inoculating various grains and grasses with *Leptosphaeria avenaria*.

Hosts	Source of inoculum <sup>1</sup>	Number of leaves	
		Inoculated	Diseased
<i>Avena barbata</i>	1	27	8
	2	8	0
	3	6	1
<i>A. brevis</i>	1	30	3
	2	6	1
	3	8	0
<i>A. fatua</i>	1	27	0
	2	7	0
	3	9	7
<i>A. nuda chinensis</i>	1	22	2
	2	9	1
	3	5	1
<i>A. sativa</i> (Culbertson)	1	14	3
	2	7	0
	3	4	2
<i>A. sativa</i> (Swedish select)	1	20	4
	2	3	1
	3	6	0
<i>A. sativa</i> (Victory)	1	27	4
	2	9	2
	3	5	2
<i>A. sativa nigra</i>	1	18	6
	2	6	1
	3	6	4
<i>A. sativa orientalis</i>	1	22	4
	2	4	1
	3	5	0
<i>A. sterilis</i> (a)	1	15	3
	2	8	2
	3	4	0

<sup>1</sup> Source of inoculum as designated in table 2 is as follows: 1. Pycnosporos from oats. 2. Pycnosporos from culture. 3. Ascospores from culture.

Hosts	Source of inoculum <sup>1</sup>	Number of leaves	
		Inoculated	Diseased
<i>A. sterilis</i> (Rust Proof)	1	19	3
	2	4	1
	3	6	2
<i>A. sterilis</i> (b)	1	29	5
	2	7	0
	3	7	3
<i>A. strigosa</i>	1	25	0
	2	15	3
	3	7	0
<i>Hordeum deficiens</i>	1	17	0
	2	9	0
	3	5	0
<i>H. horsfordianum</i> (Ore.)	1	22	0
	2	7	0
	3	4	0
<i>H. horsfordianum</i> (S. Dak.)	1	27	0
	2	11	0
	3	6	0
<i>H. jubatum</i>	1	7	0
	2	5	0
	3	6	0
<i>H. murinum</i>	1	12	0
	2	9	0
	3	6	0
<i>H. distichon erectum</i>	1	7	0
	2	7	0
	3	4	0
<i>H. distichon nudum</i>	1	11	0
	2	6	0
	3	7	0
<i>H. distichon nutans</i>	1	5	0
	2	8	0
	3	6	0

Host	Source of inoculum <sup>1</sup>	Number of leaves	
		Inoculated	Diseased
<i>H. vulgare coerulescens</i>	1	12	0
	2	7	0
	3	3	0
<i>H. v. hexastrichum</i>	1	11	0
	2	4	0
	3	5	0
<i>H. v. himalya</i>	1	9	0
	2	5	0
	3	7	0
<i>H. v. nigrum</i>	1	8	0
	2	6	0
	3	9	0
<i>H. v. pallidum</i>	1	8	0
	2	6	0
	3	6	0
<i>H. v. trifurcatum</i>	1	10	0
	2	8	0
	3	7	0
<i>Triticum aestivum</i>	1	19	0
	2	5	0
	3	4	0
<i>T. a. lutescens</i> (Harvest Queen)	1	19	0
	2	4	0
	3	6	0
<i>T. a. militura</i> (Red Wave)	1	7	0
	2	6	0
	3	6	0
<i>T. compactum</i>	1	19	0
	2	6	0
	3	7	0
<i>T. dicoccum barrum</i>	1	18	0
	2	7	0
	3	7	0



Hosts	Source of inoculum <sup>1</sup>	Number of leaves	
		Inoculated	Diseased
<i>T. d. atratum</i>	1	15	0
	2	4	0
	3	5	0
<i>T. durum</i>	1	24	0
	2	7	0
	3	4	0
<i>T. monococcum</i>	1	24	0
	2	7	0
	3	4	0
<i>T. polonicum</i>	1	20	0
	2	5	0
	3	6	0
<i>T. spelta</i>	1	19	0
	2	5	0
	3	2	0
<i>T. turgidum</i>	1	16	0
	2	3	0
	3	7	0
<i>Secale cereale</i>	1	18	0
	2	6	0
	3	3	0
<i>Agropyron repens</i>	1	16	0
	2	11	0
	3	8	0
<i>Bromus inermis</i>	1	11	0
	2	6	0
	3	7	0
<i>Chaetochloa italica</i>	1	5	0
	2	4	0
	3	8	0
<i>C. viridis</i>	1	9	0
	2	6	0
	3	4	0

Hosts	Source of inoculum <sup>1</sup>	Number of leaves	
		Inoculated	Diseased
<i>Echinochloa crus-galli</i>	1	7	0
	2	3	0
	3	5	0
<i>Panicum clandestinum</i>	1	13	0
	2	5	0
	3	7	0
<i>Pennisetum glaucum</i>	1	6	0
	2	3	0
	3	6	0
<i>Poa pratensis</i>	1	32	0
	2	10	0
	3	12	0

## MODE OF OVERWINTERING

The pycnosporos remain viable over winter when retained in the pycnidia. A number of oat leaves containing pycnidia, were collected in October, 1921, tied together with cord and fastened to a stake in the field. Every two weeks a portion of the diseased leaf was taken to the laboratory and the spores tested for percentage of germination. In every case except the collection made January 16, 1922, 90 per cent germination or above was found. On January 16, 77 per cent of the spores germinated.

## VIABILITY AND LONGEVITY OF SPORES

Pycnosporos were collected in the field in September, 1921; at that time 100 per cent of the spores germinated. Some of the diseased material was placed out of doors and on April 1, 1922, 90 per cent of the spores germinated. Some of the material was kept in a paper envelope in the laboratory. From this material 58 per cent of the spores germinated on April 1, 1922.

## MODE OF INFECTION

Seedlings for these studies were grown in the greenhouse in pots until they were about six inches high. They were then inoculated by atomizing one series with a water suspension of pycnosporos and the other with a water suspension of ascospores. Following these inoculations the plants were placed in a moist chamber for three or four days and then removed

to the greenhouse. Every 12 hours after inoculation for a six day period leaf samples were collected and placed in vials containing equal parts of 95 per cent alcohol and glacial acetic acid. They were left in this solution for from 24-36 hours, which removed the chlorophyll leaving the leaf tissue almost white. These pieces were then stained in toto with Pianze IIIb stain according to the method given by Vaughan (8). As the result of staining and clearing, the leaf tissues were light bluish-green in color and almost transparent, and the spores and mycelium were a rose red (Pl. XXX). The mycelium could be readily followed over the surface of the epidermal cells as well as in the parenchymatous tissue between the epidermal layers.

It was found that both pycnosporos and ascosporos behaved similarly in infection, namely, they lodge in the furrows between the epidermal cells and there develop germination tubes, which grow over the leaf surface. The tips apply themselves to the cuticle directly above adjoining walls of epidermal cells. The infecting hyphae penetrate the cuticle and grow between the epidermal cells and then continue between the parenchymatous cells branching in various directions. Pycnidia developed below the stomata by the hyphae collecting and matting together.

#### PERIOD OF INCUBATION

The time required for the reproduction of the disease, from inoculation to mature pycnosporos was from 12 to 16 days, averaging 14 days. The first evidence of infection appeared after 8 to 9 days, when a more or less mottling of the leaves became evident. Mature pycnidia were developed after about two weeks.

#### PATHOLOGICAL ANATOMY

The preparation of the oat leaves for the study of the pathological anatomy involved the methods previously described under "Mode of infection" except that Pianze IIIb stain was diluted with 25 per cent alcohol to one fourth strength, and the leaf tissues were left in the stain for from eighteen to thirty hours. This stained the fungus a deeper red without proportionately darkening the host tissue. It was found that when the first symptoms appeared on the leaf eight or nine days after inoculation, the fungus had extensively invaded the intercellular spaces. Strands or hyphae growing just under the epidermis had extended to the length of several epidermal cells. Sometimes it was straight but more often slightly undulating or more or less sharply bent around the parenchymatous cells. These long strands were much branched and the

branches grew in different directions between the cells limited more or less by the vascular strands of the host. The branches that extended deeper into the host tissue grew irregularly often almost completely around a single host cell. Several hyphae growing in this manner readily fill the intercellular spaces. The larger more vigorous strands of hyphae more often extended lengthwise of the leaf than crosswise or directly into the tissue. No haustoria were found. At this stage none of the cells were killed. At the end of about two weeks pycnidia were found under the stomata. They were usually directly below the stomata although occasionally they were found to be a little to one side, and always with the ostiole directly under the stomatal aperture. The pycnidia occupy all of the sub-stomatal chamber and the larger pycnidia crowd the adjoining parenchymatous cells out of place. The pycnidia were surrounded by dense mats of mycelium involving not uncommonly several adjoining parenchymatous cells. The pycnidia were in rows conforming to the rows of stomata. The invading hyphae were usually the length of four to six epidermal cells in advance of the youngest mature pycnidia. All stages in the formation of pycnidia were found, from a few clustered hyphae to the large dense mature structures. The host cells close to the pycnidia were dead and collapsed. Host cells farther away were plasmolized but still held their form while those about which only a few hyphae were growing appeared to be unchanged.

#### SUMMARY

1. The speckled blotch of oats previously reported on *Avena sativa* in France, Germany and England was found to occur to a limited extent in Wisconsin.

2. It was found only on volunteer oats, hence it was of no economic importance. The symptoms are characteristic only after pycnidia develop.

3. The ascigerous stage of the fungus has been found and belongs to the genus *Leptosphaeria*. Accordingly this species is referred to that genus and given the name *Leptosphaeria avenaria*.

4. Pycnosporos developed both on leaves of oats and in culture, and ascospores developed in culture germinated in water and on potato-dextrose agar after a few hours.

5. The cardinal temperatures for mycelial growth on agar poured plates are as follows: minimum 2° C., optimum 20 to 25° C., maximum 32° C.

6. In artificial culture the fungus grows best on an acid medium.

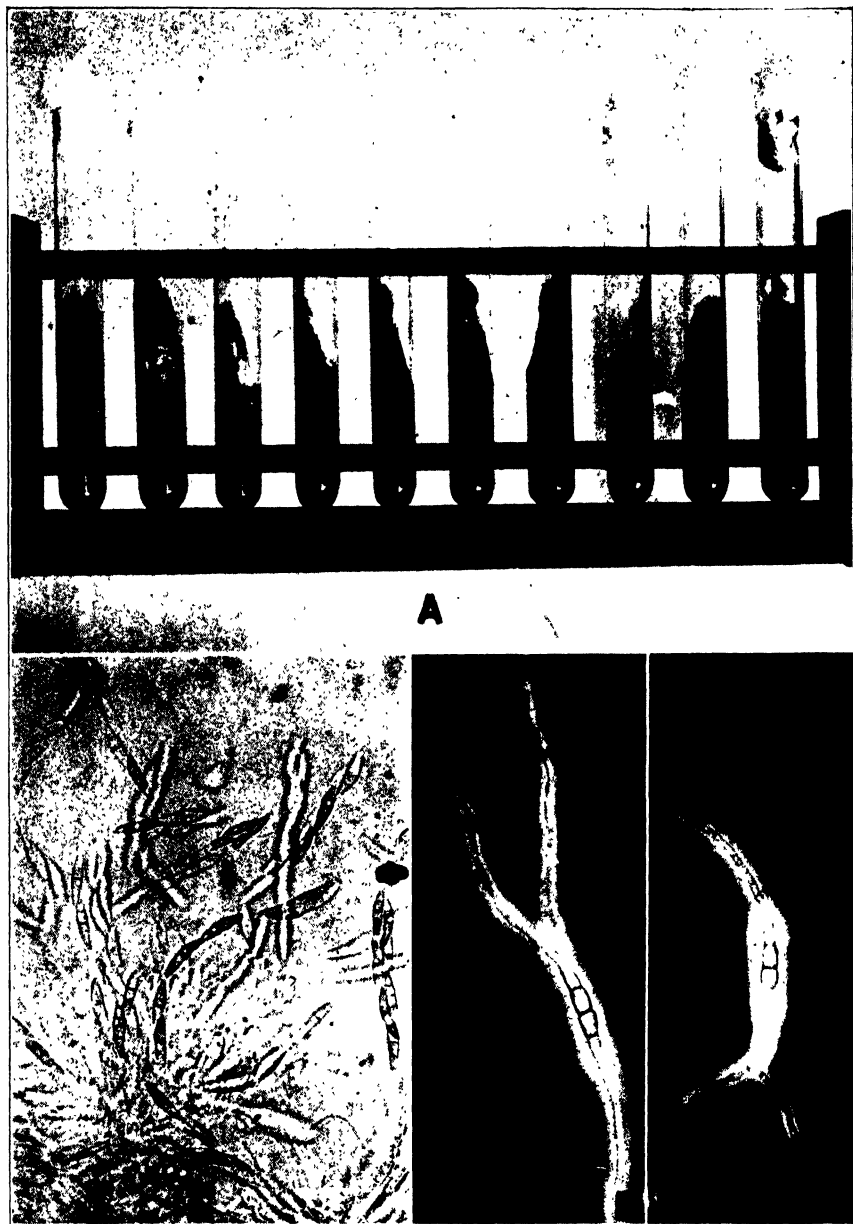
7. The fungus enters the host by direct penetration of the cuticle by germination tubes of both pycnospores and ascospores and then grows between the epidermal cells.

8. The period of incubation is from 12 to 16 days, the first symptoms appearing after eight days.

9. The mycelium grows intercellularly and the pycnidia are sub-epidermal.

#### LITERATURE CITED

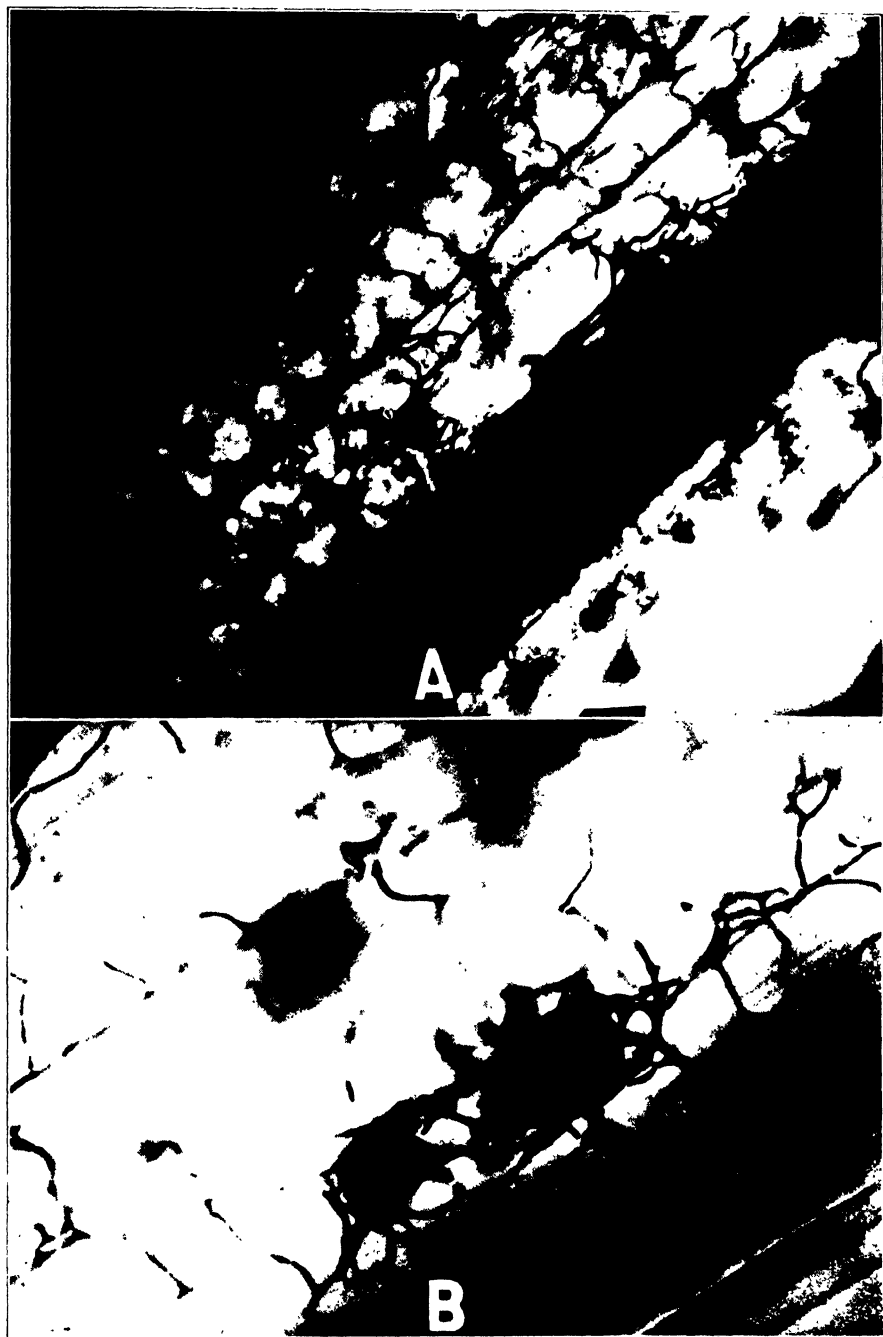
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## SPECKLED BLOTCH OF OATS

A. Potato-dextrose agar cultures in each of which pycnospores and ascospores developed. B. Photomicrographs showing germinated ascospores of *Leptosphaeria avenaria* in a medium of dilute India ink. Observe the transparent gelatinous sheath surrounding each spore and the older hyphae tapering toward the tips of the growing hyphae.  $\times 900$ . C. Photomicrograph of ascospores showing general form and characteristic habit of adhering together after coming out of the ascus.  $\times 250$ .





## SPECKLED BLOTCH OF OATS

A. Photomicrograph showing hyphae of *Leptosphaeria avenaria* in the parenchymatous tissue of an oat leaf. x 400. B. Photomicrograph showing early stage in the formation of a pycnidium of *Leptosphaeria avenaria*. X 900.





## GERMINATION OF TELIOSPORES OF RUSTS AT COLUMBIA, MISSOURI.

W. E. MANEVAL

The teliospores of rusts generally germinate only after a more or less definite resting period, usually extending from summer or autumn of one year to the time of leaf development of the host the following year. The period of rest may be quite short, while there are numerous leptotypes and some others that require no resting period. Plowright (18) indicated what is true in this respect for many of the species listed in his monograph. Dietel (3) has published a rather long list of leptotypes and of some others whose teliospores germinate soon after maturity. These include eu-, brachy-, and hemi-types. He states that leptotypes are rare on Gramineae, Cyperaceae and Umbelliferae. Klebahn (13) says it is generally necessary for the teliospores of heteroecious rusts to pass the winter in the open. Eriksson (8) succeeded in germinating teliospores of *Puccinia graminis* after keeping them one or two winters in an herbarium and then one winter in the open. Woronin (24) found that teliospores of *Puccinia helianthi* would germinate equally well after passing the winter in the open or dry indoors, while Carleton (2) discovered that they would germinate to some extent in October without passing through a resting period. Klebahn (13, p. 12) divides heteroecious rusts into 6 developmental types. In one type the teliospores germinate only after a winter rest period (most species of *Uromyces* *Puccinia*, *Melampsora*, and *Pucciniastrum*), while the other 5 types include forms that hibernate as aecial or telial mycelium or whose method of hibernation is unknown. In these 5 types the teliospores germinate immediately after maturity.

During the past 5 years the writer has made observations at various dates between fall and spring on the germination of teliospores of a number of species of rusts occurring at Columbia, Missouri. The following species were used in the germination tests:

*Phragmidium potentillae-canadensis* Diet. on *Potentilla canadensis* L.

*Puccinia asparagi* DC. on *Asparagus officinalis* L.

*Puccinia helianthi* Schw. on species of *Helianthus*, mainly *Helianthus strumosus* L. and *Helianthus hirsutus* Raf.

*Puccinia menthae* Pers. var. *americana* Burr. on *Monarda fistulosa* L.

*Puccinia ruelliae* (B. & Br.) Lagh. on *Ruellia strepens* L.

*Puccinia andropogonis* Schw. on *Andropogon furcatus* Muhl.

*Puccinia peridermiospora* (Ell. & Tracy) Arth. on *Spartina michauxiana* Hitch.

*Puccinia sorghi* Schw. on *Zea mays* L.

*Puccinia sydowiana* Diet. on *Sporobolus asper* (Michx.) Kunth.

*Puccinia windsoriae* Schw. on *Tridens flavus* (L.) Hitch.

The tests show that all of these forms may germinate at the beginning of winter (December) and in some cases earlier. These rusts are all eu-types. The first 5 are autoecious and the last 5 heteroecious. The data presented serve as records of germination of these rusts at a definite station and have a bearing on conditions for germination. The records for 4 of the species are for one year only, while in the other cases they are for two to four years. Some of the factors especially concerned in the germination of teliospores of rusts are moisture, temperature, oxygen supply, and maturity or age of the spores, while light, substratum and certain other conditions seem to be of minor importance. Some effects of these factors will be briefly reviewed.

*Moisture.* Generally a practically saturated atmosphere is necessary for germination. The amount of moisture present influences germination, morphology and production of sporidia. Blackman (1) found that teliospores of *Puccinia graminis*, *Uromyces fabae* and *Phragmidium rubi* immersed in water developed long promycelia but no sporidia until they grew out into the air; however if not immersed they produced characteristic promycelia and sporidia. Weimer (23) also got long promycelia of *Gymnosporangium juniperi-virginianae* in water, but if little moisture was present the usual type of promycelia bearing sporidia developed. Melhus, Durrell and Kirby (17) found that teliospores of *Puccinia graminis* attached to pieces of straw would not germinate if the humidity of the atmosphere was less than 96.5 per cent.

The effect of drying and especially of alternate wetting (soaking) and drying of teliospores has been noted repeatedly. Dietel (4, vol. 31) found that drying decreased markedly the time (from three days to two and three-fourths hours) necessary for germination to begin in the case of *Melampsora larici-caprearum* Kleb. In other experiments (4, vol. 54) he collected teliospores of the same rust October 31, 1915, and kept them dry until November 4 and 13. On these dates he subjected the spores to alternate wetting and drying for periods of two or three days until December 21, when they would all germinate, there being no difference between the two sets. In 1916 spores of this rust were collected November 11 and after three soakings germinated abundantly December 16. The same result was secured in 1917, with spores collected November 22, and soaked five to seven times. Dietel concludes that teliospores

are not in an unchangeable condition and that germination may be brought about earlier than normally by alternate wetting and drying.

In certain germination tests in 1911, with *Puccinia graminis* Pers. on *Agropyron repens* P. De Beauv., *Puccinia phragmites* (Schum.) Korn. on *Phragmites communis* Trin. and *Puccinia magnusiana* Korn. on *Phragmites communis*, Klebahn (14) found that teliospores kept dry in the open, exposed to the cold and dampness of the air but not rain, did not germinate May 1. Tests resulted negatively also with spores kept in a dry room over winter; but spores of *Puccinia graminis* and *Puccinia phragmites* kept alternately wet and dry (three day periods) from November 7, to April 24, with the temperature always above 0° C. germinated abundantly May 1.

Similar results were obtained by Klebahn in 1912, after subjecting spores to flowing water and to alternate wetting and drying for three and eight day periods. The experiments were started with *Puccinia phragmites* November 13, and with *Puccinia graminis* November 29. Spores kept alternately wet and dry and tested January 10 germinated quite well. Controls of *Puccinia graminis* kept in a warm room germinated slightly while those of *Puccinia phragmites* did not germinate. Klebahn concludes that the most important factor for germination is alternate saturation with water and drying. Changing the water proved important also and this, he suggests, may be in connection with the oxygen content. Winter cold was not necessary, but seemed to have an accelerating influence on germination.

*Temperature.* Many more records are available regarding the relation of temperature to germination of urediniospores than of teliospores. The work of Johnson (12), Ward (22), Gibson (10), Mains (16), Doran (5), Howell (11) and Durrell (6) shows that for nearly twenty species of rusts the cardinal temperatures for germination of urediniospores are almost without exception between the following limits: minimum 2° C. to less than 10° C.; optimum, 12° C. to 20° C.; maximum, 25° C. to less than 30° C. Compared with cardinal temperatures for germination of spores of other fungi these are rather low, and it seems that the same holds true for aeciospores and teliospores. Doran (5) reports 5°, 12° and 19° C. as cardinal temperatures for germination of aeciospores of *Cronartium<sup>1</sup> ribicola* and 9°, 16° and 29° C. for aeciospores of *Gymnosporangium clavipes*.

Dietel (4, vol. 35) states that he found no influence of temperature on germination of teliospores of *Melampsora larici-capreae* between 8° C. and 22° C. and secured good germination even at 26° C. He concluded for *Puccinia graminis* that the minimum temperature for

germination is probably about 9.5° C., and normal germination occurred at 22° C. At 23° C. and higher the promycelia were abnormal and produced neither sterigmata nor sporidia. Others have reported cardinal temperatures for germination of teliospores of rusts as follows:

<i>Puccinia malvacearum</i>	3°	14°	30° C.	Doran (5)
<i>Puccinia graminis</i>	5°	20°	30° C.	Melhus (17) No sporidia above 25° C.
<i>Puccinia helianthi</i>	20—°	24°±	28+° C.	Fraizer (9)
<i>Gymnosporangium juniperi-virginianae</i>	11.5°	15°	29° C.	Reed & Crabill (19) No sporidia above 24° C.
<i>Gymnosporangium juniperi-virginianae</i>	7°	22–25°	29° C.	Weimer (23)
<i>Gymnosporangium globosum</i>	7°	22–25°	29° C.	Weimer (23)
<i>Gymnosporangium clavipes</i>	7°	22–25°	29° C.	Weimer (23) Some sporidia at 26° C.

These temperatures correspond in general with the cardinal temperatures for germination of urediniospores and aeciospores. The germination of spores of various fungi is favored by exposure to low temperature, but that this is unnecessary for germination of teliospores of various rusts has been demonstrated repeatedly. McAlpine (15) kept samples of teliospores of *Puccinia graminis* on badly rusted straw at 4° C. and—18° C. for three months and also in the open in Australia. When tested in the spring only those kept in the open germinated (p. 24). He also states (p. 66) that, "seeing that the spores (teliospores of *Puccinia graminis*) germinated freely here, the exposure to cold theory does not hold." He also says (p. 9) that germination tests in Victoria during several seasons resulted positively only from September to November (spring) after a resting period of about eight months including the hot and dry summer.

*Chemicals.* No one, it seems, has reported improvement in germination of teliospores by using complex nutrient solutions. It is likely that oxygen is absolutely necessary for germination. Reed and Crabill (19) failed to secure germination of *Gymnosporangium juniperi-virginianae* in an atmosphere of carbon dioxide and state that oxygen is apparently essential. Dietel (4, vol. 54) tested teliospores of *Puccinia graminis*, *Melampsora larici-caprearum* and *Melampsora larici-tremulae* in an atmosphere of carbon dioxide, in air with the oxygen removed, and in a vacuum. When kept under such conditions for 4, 24 and 48 hours, the spores did not germinate. These spores were then put in air and germination occurred in every case as well as in all controls.

Thiel and Weiss (21) treated telial material of *Puccinia graminis*

collected August 12, 1919, in Minnesota with 1 per cent citric acid for five minutes to one hour and on testing secured from one to fifteen per cent of germination with spores treated fifteen or twenty minutes. The spores were exposed to out-door conditions until the time of testing (December 23 and 29; January 14 and 26; February 21), but there was little difference in the percentage of germination on these different dates. Treatments with m/100 HCl, m/100 to m/5 H<sub>2</sub>SO<sub>4</sub>, 1 per cent H<sub>3</sub>PO<sub>4</sub>, 1, 2.5 and 5 per cent lactic and acetic acids, 0.0625 to 5 per cent H<sub>2</sub>O<sub>2</sub>, etc., resulted negatively. It seems possible that with the acid treatments in most cases the concentration may have been too high to hope for a favorable influence.

*Maturity.* If we regard the changes that occur in teliospores during the winter as a maturing process this may explain in a way several phenomena connected with germination. All of the spores in a pustule do not germinate at one time but during a considerable period of time. Reed and Crabill (19) found that teliospores of *Gymnosporangium juniperi-virginianae* germinated after every considerable rain from April 1 to June 1, those on the outside of the tentacle germinating first. McAlpine (15) makes similar statements regarding *Puccinia graminis* (p. 9 & 24). Eriksson (7) was of the opinion that only the crop of teliospores of *Puccinia graminis* maturing in late autumn is able to germinate the following spring. As spring approaches the percentage of spores that will germinate in a given species requiring a rest period increases gradually to a maximum and then declines. Moreover, it has been noted by certain observers that as the time of maximum germination approaches the time necessary for germination to start decreases, sometimes to an hour or less. Dietel (4, vol. 35) germinated the teliospores of *Puccinia graminis* at the middle of March in central Germany and found that the time necessary for germination to begin decreased gradually from thirty hours to two or two and three-fourths hours, and then increased until practically no more germination occurred July 1. He found also that spores of *Puccinia malvacearum* began to germinate in fifteen minutes, the formation of sporidia began in two hours, and in some cases was complete in two and one-half hours.

#### EXPERIMENTAL

After collecting telial material it was kept at room temperature (20°± C.) or in a few cases, as stated below, in a cold room somewhat above 0° C. In making germination tests the teliospores were floated on the surface of distilled water in a preparation dish of 30 cc. capacity. The amount of water used varied between 10 and 15 cc. The dishes were

kept covered and at room temperature ( $20 \pm ^\circ \text{C.}$ ). It was difficult to determine accurately the percentage of germination so generally the amount of germination is indicated approximately as follows: 0 = none; + = few spores to less than 1 per cent germinating; ++ = 1-5 per cent; +++ = 10  $\pm$  per cent or good; ++++ = 30-90 per cent or very good to excellent. Wherever approximate percentages are given they were obtained by removing carefully a part of the spore suspension to a drop of water on a slide and then counting the number of germinated and ungerminated spores in several fields of the microscope. Most of the tests were made in duplicate and the cultures were examined every day or more frequently for several days, and after that at intervals of two or three days. Examinations for germination could readily be made with the low power of the microscope without removing spores from the culture dish.

In the tables that follow the principal results of the tests are given. With certain forms, particularly *Puccinia helianthi*, *Puccinia peridermiospora* and *Puccinia windsoriae* many more tests were made than are presented in detail here.

#### PUCCINIA ASPARAGI

Only one test was made with this rust. Spores collected December 27, 1921, and tested the same day began to germinate in 6 days or less, and during the next 10 days approximately 5 per cent germinated. Germination in m/15  $\text{KH}_2\text{PO}_4$  (pH 4.6)<sup>1</sup> and in m/15  $\text{KH}_2\text{PO}_4$  plus m/15  $\text{K}_2\text{HPO}_4$  (pH 5.4) was decidedly poorer than in water.

Smith (20) did not succeed in germinating teliospores of this rust in California between June 20 and December 12, using material collected between June 8 and November 19. He did succeed, however, in germinating a few spores collected December 1 and tested December 12. The germinating capacity increased as the season advanced to February 26 (last test).

#### PUCCINIA SYDOWIANA

In a test of spores collected December 27, 1921, and started two days later germinating spores were observed at the end of 22 days and the percentage of germination increased to between 2 and 5 per cent in the next 3 weeks.

Spores of the same collection were also frozen in ice the night of January 6, and then kept dry in a cold room until January 13. They

<sup>1</sup>Hydrogen-ion concentration in all cases was determined colorimetrically by Gillespie's method.

were then frozen in ice for four successive nights, January 13 to 16, after which they were dried and tested January 18. In this test the time for germination to begin was decreased and in 3 weeks a higher percentage (5 to 10 per cent) had germinated than during 6 weeks in the preceding test.

#### PUCCINIA SORGHI

This rust was collected December 27, 1921, and tested December 29. Germination began in less than 2 days and increased from day to day until most of the spores had germinated at the end of 11 days.

Another test was made January 13, with spores from the same collection as used in the previous one. The spores had been kept in a cold room in the meantime and germinated as well or better than when tested soon after collection.

Germination was retarded in m/15  $\text{KH}_2\text{PO}_4$  (pH 4.6) and in a mixture of m/15  $\text{KH}_2\text{PO}_4$  and m/15  $\text{K}_2\text{HPO}_4$  (pH 5.4) during the early part of the germination period, but finally (11 days) as large a percentage of spores had germinated as in water. These facts are summarized in table 1.

Mains (16) states that the teliospores of *Puccinia sorghi* require wintering before germinating. However, he collected fresh material in the greenhouse in January and after alternate soaking and drying for 3 day periods repeated 5 times secured fair germination.

TABLE 1

*Germination of teliospores of Puccinia sorghi*

Collected	Tested	Incubation period	Result	Remarks
12/27/21	12/29/21	2 days	5-10 per cent	
"	"	5 "	50± per cent	
"	"	11 "	90± per cent	
"	"	5 "	20-30 per cent	In phosphates, pH 4.6 and 5.4.
"	"	11 "	90± per cent	In phosphates, pH 4.6 and 5.4.
"	1/13/22	3 "	50± per cent	Had been kept in cold room.

#### PUCCINIA RUELLIAE

With this rust it will be noted (Tab. 2) that the time required for considerable germination of the spores varies with the date of testing, being less than 2 days in May, less than 8 days in December and decidedly longer in September. Also, as with the preceding species, the percentage of germination may increase gradually over a considerable period of



time, as is seen in the test begun September 13, 1918, in which a very high percentage of spores germinated in 53 days.

TABLE 2

*Germination of teliospores of Puccinia ruelliae*

Collected	Tested	Incubation period	Result	Remarks
10/23/16 <sup>1</sup>	4/5/17	5 days	0	
"	"	8 "	+ +	
"	5/7/17	43 hours	+ + +	
9/10/18	9/13/18	39 days	+ + +	
"	"	53 "	+ + + +	
"	10/ 3/21	32 "	+ + +	Wet and dry, 3 day periods, 9/13 to 10/3.
12/27/21	12/29/21	8 "	5 per cent	

Acid reaction (phosphates, pH 4.6 and 5.4) retarded germination as was the case with *Puccinia sorghi* and *Puccinia asparagi*.

## PHRAGMIDIUM POTENTILLAE CANADENSIS

Only two collections of this rust, made in the fall of 1917, were tested. Germination of the spores in each case began in from 2 to 5 days and was practically complete in 20 days in the later collection and in 28 days in the earlier one. Again after germination began (2 to 5 days) it extended over periods of 3 to 4 weeks.

TABLE 3

*Germination of teliospores of Phragmidium potentillae canadensis.*

Collected	Tested	Incubation period	Result
10/7/17	11/3/17	5 days	+
"	"	12 "	+ +
"	"	28 "	100— per cent
10/16/17	10/19/17	15 "	+ + +
"	"	20 "	100— per cent
"	11/3/17	2 "	+
"	"	7 "	+ +

## PUCCINIA MENTHAE VAR. AMERICANA

Teliospores collected November 22, 1921, germinated fairly well in 15 days, and approximately fifty per cent from the collection of December 27, germinated in 3 days. This time was still shorter (1½ days) in

<sup>1</sup> All rusts collected October 23, 1916, were put in muslin bags and kept out of door during the winter and were partially protected from rain and snow.

tests made March 10, 1920. Two tests with solutions of phosphates (pH 4.6 and 5.4), using spores collected December 27, 1921, resulted in very marked inhibition of growth during the first 3 days of the test, with considerable inhibition in the less acid solution after 6 days and marked inhibition in the more acid one. It was found, also, that this rust and certain others might be kept floating on water at temperatures above the maximum for germination for 2 or 3 days and then, if changed to a temperature near the optimum, they would germinate.

TABLE 4

*Germination of teliospores of Puccinia menthae var. americana*

Collected	Tested	Incubation period	Result	Remarks
10/23/16	4/5/17	3 days	+ + + +	
"	4/16/17	67 hours	0	28° C.; then 25 hours longer at room temperature + + +.
1/5/18	1/7/18	3 days	+ + + +	
3/9/20	3/10/20	1½ "	+ + + +	
11/22/21	11/22/21	15 "	+ + +	
12/27/21	12/27/21	6 "	80 per cent	
"	12/29/21	2 "	20-30 per cent 3 days, 50± per cent	
"	12/27/21	3 "	5—per cent m/15 phosphate, pH 4.6 and 5.4 per cent	
"	"	6 "	10 per cent m/15 phosphates, pH 4.6; 50 ± per cent with pH 5.4	

#### Puccinia WINDSORIAE

Teliospores of this rust collected December 17, 1918, germinated somewhat in 35 days and those collected December 27, 1921, germinated slightly in 10 days while 5 ± per cent germinated in 41 days. Similar results were obtained with material collected January 5, 1918, and January 9, 1922. Spores collected October 16, 1917, failed to germinate in 61 days, but when kept till January and then subjected to alternate wetting and drying for two weeks they germinated slightly in 17 days. The time for germination however, decreased to two days for spores collected in April, 1918 and 1922.

The percentage of spore germination with this rust was generally not high. Alternate wetting and drying in the case of collections made January 15, 1918, and February 26, 1918, seemed to improve the germination and to shorten the time necessary for it to begin. Spores collected April 30, 1918, did not germinate in two days at either 28° C. or 32° C. although they did germinate to some extent when kept two

days longer at room temperature. Most of the facts mentioned are summarized in table 5.

TABLE 5

*Germination of teliospores of Puccinia windsoriae*

Collected	Tested	Incubation period	Result	Remarks
2/26/18	2/27/18	12 days	0	
"	3/7/18	4 "	0	1 day wet, 3 days dry, 2/27-3/7
"	3/11/18	4 "	+	1 day wet, 3 days dry, 2/27-3/11
"	3/15/18	2 "	++	1 day wet, 3 days dry, 2/27-3/15
4/30/18	4/30/18	2 "	++	Had germinated in part out of doors
10/16/17	10/16/17	61 "	0	
"	1/28/18	17 "	+	3 days wet, 3 days dry, 1/7 to 1/28
"	2/23/18	9 "	++	3 days wet, 3 days dry, 1/7 to 2/23
9/10/18	9/13/18	36 "	0	
12/17/18	12/17/18	35 "	+++	
1/5/18	1/7/18	31 "	+	Much better in 39 days,
"	1/28/18	21 "	++	3 days wet, 3 days dry, 1/7 to 1/28
"	1/30/18	8 "	++	1 day wet, 2 days dry, 1/15 to 1/30
"	2/11/18	3 "	+++	1 " wet, 2 " dry, 1/15 to 2/11
12/27/21	12/29/21	10 "	+	5± per cent in 41 days.

## PUCCINIA ANDROPOGONIS

Alternate wetting and drying improved the germination of this rust. Spores from collection made December 17, 1918, failed to germinate floating on water in 35 days, but they germinated quite well in 6 days after alternate wetting and drying from December 17 to January 26 (3 day periods). Spores collected January 5, 1918, also failed to germinate in water in 39 days but two cultures germinated somewhat in 3 and 7 days after alternate wetting and drying from January 7 to February 11 and 13 respectively. Also teliospores collected February 26, 1918, did not germinate in water in 12 days but when kept alternately wet and dry (1 day wet and 3 dry) and tested March 7, 11 and 15 some spores germinated in 1 or 2 days and in the case of the last test 20 to 25 per cent germinated in 3 days (Table 6).

That teliospores of this rust may germinate as early as December was determined by tests made with material collected December 27, 1921, and tested December 29, when numerous spores germinated in 2 days. However very few more spores germinated in these cultures when kept 16 days longer. Spores of this same collection were frozen in ice January 6, dried, soaked in water January 9, dried and tested January 10. In the course of 3 days about 5 per cent germinated.

TABLE 6

*Germination of teliospores of Puccinia andropogonis*

Collected	Tested	Incubation period	Result	Remarks.
12/17/18	12/17/18	35 days	0	
"	1/26/19	6 "	+ + +	3 days wet, 3 days dry, 12/17 to 1/26.
1/5/18	1/7/18	39 "	0	
"	1/28/18	17 "	0	3 days wet, 3 days dry, 1/7 to 1/28.
"	2/11/18	3 "	+	3 days wet, 3 days dry, 1/7 to 2/11
1/15/18	1/30/18	15 "	0	1 day wet, 2 days dry, 1/15 to 1/30.
"	2/11/18	3-14 "	+	1 day wet, 2 days dry, 1/15 to 2/11.
2/26/18	2/27/18	12 "	0	
"	3/7/18	2-6 "	+	1 day wet, 3 days dry, 2/26 to 3/7.
"	3/11/18	4 "	5 per cent	1 day wet, 3 days dry, 2/26 to 3/11.
"	3/15/18	3 "	20-25 per cent	1 day wet, 3 days dry, 2/26 to 3/15.
3/9/20	3/10/20	23 "	0	
"	4/8/20	2-4 "	+ +	
12/27/21	12/29/21	2, 5, 18 "	+	
12/28/21	1/10/22	3 "	5 per cent	Frozen in ice 1/6; soaked 1/9.

## PUCCINIA PERIDERMIOSPORA

Teliospores of this rust collected October 16, 1917, and September 28, 1918, failed to germinate in 61 and 38 days respectively. Spores collected December 17, 1918, and January 5, 1918, germinated quite well after 35 and 32 days. The time for germination to begin or to reach a high percentage gradually decreased between December and April as with other rusts. This is seen too on comparing dates much closer together; for example, while the collection of March 19, 1917, required 10 days for slight germination considerable germination occurred in the collection of April 6, 1917, in 2 days. The effect of alternate wetting and drying is illustrated by the collection of February 26, 1918, which required 10 days for slight germination but germinated very well ( $40 \pm$  per cent) in from 2 to 5 days after alternate wetting and drying between February 27 and March 20. It seems too that considerable change may occur even though the spores are kept dry at room temperature, for while the spores collected March 9, 1920, germinated somewhat after 15 days those tested April 8 germinated very well in 6 days. This is also illustrated by the behavior of other species. Spores of *Puccinia andropogonis* collected March 9, and tested March 10, 1920, failed to germinate in 23 days but some germination occurred in from 2 to 4 days when the spores were tested April 8.

TABLE 7.

*Germination of teliospores of Puccinia peridermiospora*

Collected	Tested	Incubation period	Result	Remarks
3/19/17	3/20/17	10 days	+	
4/6/17	4/7/17	2 "	+ + +	
5/5/17	5/7/17	3 "	0	Apparently germinated out-doors.
12/17/18	12/17/18	35 "	+ + + +	Not examined till 1/21/19.
"	1/26/19	6 "	+ + +	3 days wet, 3 days dry, 12/17 to 1/26.
1/5/18	1/7/18	32 "	+ + +	
"	1/28/18	10 "	+ +	3 days wet, 3 days dry, 1/7 to 1/28.
"	2/11/18	2-5 "	+ +	3 days wet, 3 days dry, 1/7 to 2/11.
1/15/18	1/30/18	8 "	+ + +	1 day wet, 2 days dry, 1/15 to 1/30.
2/26/18	2/27/18	10 "	+	
"	3/11/18	1 "	+ +	1 day wet, 3 days dry, 2/27 to 3/11.
"	3/20/18	2 "	+ + +	1 day wet, 3 days dry, 2/27 to 3/20.
"	"	5 "	40 $\pm$ per cent	1 day wet, 3 days dry, 2/27 to 3/20.
3/13/18	3/13/18	5 "	0	
3/9/20	3/10/20	15 "	+ +	
"	4/8/20	6 "	+ + + +	
4/30/18	4/30/18	1 "	+ +	
12/27/21	12/27/21	18 "	0	
12/28/21	2/7/22	13 "	10 $\pm$ per cent	20 days, 100— per cent in good part.

## PUCCINIA HELIANTHI

Woronin (24) collected teliospores of this rust in the fall (date not given) and kept them wrapped in paper in a room. Nearly 100 per cent germinated in 2 days, February 3, and spores tested between February and the middle of May germinated very well, regardless of preservation at room temperature or covered with snow all winter. Gradually the power to germinate was lost so that tests made July 19, resulted negatively at the end of 3 days, and only a few spores germinated in 5 days.

Many tests were made with *Puccinia helianthi* but most of the results were similar to those given in table 8. Spores collected in October would germinate slightly in two months or more. Spores collected October 7, 1917, germinated only slightly in 70 days but when kept 43 days longer germinated very well, illustrating the effect of continued floating on water. Spores collected December 2, 1916, germinated somewhat in 3 days. From 5 to 40 per cent of the spores collected December 27, 1921, germinated in 6 to 11 days, but only a few spores of a collection made November 22, 1921, germinated in 14 days. Results of tests with spores collected January 5, 1918, were similar to those for December 27, 1921, but practically 100 per cent of spores collected February 26, 1918, germinated in 5 days while a very high percentage

of spores collected April 6, 1917 and 1921, would germinate in one day. Germination in the latter case began in less than an hour and some sporidia were produced within two hours.

Spores collected October 11, 1916, and kept in an herbarium till April 18, 1917, germinated somewhat in 38 days, and also spores collected December 2, 1916, and kept as herbarium material germinated in 2 days, March 7, 1917. This indicates that the date of collection in the fall or early winter may influence germination after keeping over winter in a warm room. But that neither a rest period nor low temperature is necessary for germination is indicated by a test of spores from a living plant in the greenhouse collected March 12, 1917, the spores of which germinated to some extent in from 6 to 8 days.

Teliospores of *Puccinia helianthi* would germinate feebly at 28° to 29° C. but the promycelia were abnormal and practically no sporidia were formed. Spores of *Puccinia windsorise*, *Puccinia peridermiospora* and *Puccinia helianthi* would not germinate at 32° C. but when removed to room temperature gave positive results. Spores of *Puccinia helianthi* after floating on water at 38° C. for 48 hours failed to germinate, but withstood drying for 5 days at 38° C. and still germinated. Temperatures above the maximum delayed the time for germination to begin without inhibiting it completely; for example, spores collected April 6, 1917, and tested April 23, germinated well (+++++) in one day at room temperature but failed to germinate in 52 hours at from 32° to 33° C. However, when this culture was removed to room temperature some germination (++) occurred in 24 hours.

Spores collected December 27, 1921, were tested in water, in m/15  $\text{KH}_2\text{PO}_4$  (pH 4.6) and in a mixture of m/15  $\text{KH}_2\text{PO}_4$  and  $\text{K}_2\text{HPO}_4$  (pH 5.4). At the end of three days 1 ± per cent had germinated in water and 10 per cent in the solutions. At the end of 7 days the corresponding results were 5 to 10 per cent and 30 to 40 per cent.

Tests of spores collected April 6, 1922, were made April 10 to determine whether the concentration of  $\text{KH}_2\text{PO}_4$  might influence germination, 1.25, 0.75, 0.50, 0.20, 0.10 and 0.05 per cent solutions and water being used. At the end of 3 hours inhibition was evident with the three most concentrated solutions. After 20 hours there was still marked inhibition in the strongest solution and possibly a little in the 0.75 per cent solution. An experiment was then performed April 11, with spores collected April 6, 1922, using first, 0.2 per cent  $\text{KH}_2\text{PO}_4$ , second a mixture of 0.2 per cent  $\text{KH}_2\text{PO}_4$  and 0.05 per cent  $\text{H}_2\text{PO}_4$ , third 0.2 per cent  $\text{KH}_2\text{PO}_4$ , and 0.25 per cent  $\text{K}_2\text{HPO}_4$ , and fourth 0.2 per cent  $\text{KH}_2\text{PO}_4$  and n/20

NaOH. The solutions had the hydrogen-ion concentration indicated below where the results are shown for 1, 2.5 and 19.5 hours.

pH	3.5	3.9	4.7	4.9	5.4	5.6	6±	6.5	7±	7.8	7.9	8.1	8.3	8.7±	Hr.
Germination	0	0	+	+	+	+	+	+	+	0	0	0	0	0	1
Germination	oc	oc	g	g	g	g	g	g	g—	g—	oc	oc	oc	vf	2.5
Germination	vf	f	vg	vg	vg	vg	vg	vg	g	g	f	vf	vf	oc	19.5
Sporidia	vf	vf	vg	vg	vg	vg	fr	fr	f	oc	oc	0	0	0	19.5

0= none; += germination; oc= occasional spore; g= good; vg= very good; f= few; fr= fewer; vf= very few.

The H-ion concentration of the solutions had not changed at the end of 20 hours excepting that the index for the last was 8.4. This experiment indicates that for *Puccinia helianthi* the limits of H-ion concentration for germination of teliospores are represented approximately by pH 3.5 and 8.4 and for production of sporidia by pH 3.5 and 7.8. Good or excellent germination occurred between concentrations represented by pH 4.6 and 7, while good sporidia production occurred within narrower limits (pH 4.6–6.5).

TABLE 8.

*Germination of teliospores of Puccinia helianthi.*

Collected	Tested	Incubation period	Result	Remarks
10/11/16	4/18/17	38 days	+	Kept in herbarium during winter.
12/2/16	3/7/17	2 "	+	Kept in herbarium during winter.
"	12/9/16	3 "	+	
10/7/17	10/10/17	70 "	+	103 days, + + + +
10/16/17	11/10/17	39–56 "	+	
3/12/17	3/12/17	6–8 "	+	From living plant in greenhouse.
4/6/17	4/7/17	1 "	+	
"	4/23/17	1 "	+	1 hour, + +
"	4/28/17	2 "	+	Dried 5 days at 38° C.
5/5/17	5/7/17	1 "	+	Most spores had germinated out-doors.
10/22/18	10/22/18	4 "	+	
1/5/18	1/7/18	7 "	+	50+ per cent.
"	1/12/18	3 "	+	90 per cent; 3 days wet, 2 day dry, 1/7–1/12
2/26/18	2/27/18	1 "	+	2 days + + +; 5 days, 100 per cent.
4/11/18	4/12/18	19 hours	+	
11/22/21	11/22/21	14 days	+	
12/10/21	12/27/21	6 "	1 + per cent	In m/15 KH <sub>2</sub> PO <sub>4</sub> (pH 4.6) 20+ per cent.
12/27/21	"	6 "	5–10 "	In m/15 KH <sub>2</sub> PO <sub>4</sub> (pH 4.6) 30–40 per cent.
"	"	11 "	40 "	In m/15 KH <sub>2</sub> PO <sub>4</sub> (pH 4.6) 40 per cent.

## DISCUSSION AND SUMMARY

These records for germination of teliospores of 10 species of rusts collected and tested at Columbia, Missouri, show that they all germinated in December or earlier as follows:

TABLE 9.

*Germination of teliospores of rusts.*

	Collected	Tested	Incubation period	Result	Remarks
<i>Phragmidium potentillae-canadensis</i>	10/7/17	11/3/17	5 days	+	Not tested earlier
<i>Puccinia andropogonis</i>	12/27/21	12/29/21	2 "	+	
<i>Puccinia asparagi</i>	12/27/21	12/27/21	6 "	+	Not tested earlier
<i>Puccinia helianthi</i>	10/ 7/17	10/11/17	70 "	+	
	10/22/18	10/22/18	4 "	+	
<i>Puccinia menthae</i>	11/22/21	11/22/21	15 "	+ + +	
<i>Puccinia peridermiospora</i>	12/17/18	12/17/18	35 "	+ + + +	
<i>Puccinia ruelliae</i>	9/10/18	9/13/18	39 "	+ + +	
<i>Puccinia sorghi</i>	12/27/21	12/29/21	5 "	50 $\pm$ per cent	Not tested earlier
<i>Puccinia sydowniana</i>	12/27/21	12/27/21	22 "	+	
<i>Puccinia windsoriae</i>	12/17/18	12/17/18	35 "	+ + +	

In 4 cases tests were not made earlier than the dates given. It seems probable that in the case of 3 of these species (*Phragmidium potentillae-canadensis*, *Puccinia asparagi* and *Puccinia sorghi*) germination would have occurred considerably earlier.

As the season advances there is a decided increase in the percentage of spores that will germinate in a given time. For example, teliospores of *Puccinia helianthi* required 103 days for a high percentage of germination in October, 11 days in December, 7 days in January, 5 days in February and less than one day in April. Some of the other species (*Puccinia menthae*, *Puccinia peridermiospora*, *Puccinia windsoriae*) behaved similarly. For the remaining forms the data were not sufficient to determine this point.

The time required for germination to begin decreases as spring approaches. This point is well illustrated by the behavior of *Puccinia helianthi*. Spores of this rust germinated slightly in 70 days October 10, 1917, but better in 6 days December 27, 1921, in one day February 27, 1918, and in one or two hours in April 1917 and 1922. The data presented indicate that the same is true for *Puccinia peridermiospora*, *P. windsoriae*, *P. menthae* and *P. ruelliae*. A small percentage of spores of *P. peridermiospora* germinated in 32 days January 7, 1918, in 15 days March 10, 1920, and in 2 days April 7, 1917. Spores of *P. windsoriae* failed to germinate in 36 days September 13 and in 61 days October 16, 1918, but a small percentage germinated in 41 days December 29, 1921, in 35 days December 17, 1918, in 39 days January 7, 1918, and in 2 days



April 30, 1918. From 1 to 5 per cent of spores of *P. menthae* germinated in 15 days November 22, 1921, but 20 to 30 per cent germinated in 2 days December 29, 1921.

After germination in a culture has begun it will generally continue for a considerable period of time. This may be seen in the following table.

TABLE 10

*Effect of length of incubation period on percentage of germination of teliospores.*

	Tested	Incubation period	Result	Incubation period	Result
<i>Phragmidium potentillae-</i> <i>canadensis</i>	11/3/17	5 days	+	28 days	100—per cent
<i>Puccinia asparagi</i>	12/27/21	6 "	+	17 "	5± per cent
<i>Puccinia helianthi</i>	2/27/18	1 "	+	5 "	100—per cent
<i>Puccinia menthae</i>	12/29/21	2 "	20-30 per cent		
	12/27/21			6 "	80 per cent
<i>Puccinia peridermiospora</i>	2/7/22	13 "	10 per cent	20 "	100—per cent
<i>Puccinia ruelliae</i>	9/13/18	39 "	+++	53 "	++++
<i>Puccinia sorghi</i>	12/29/21	2 "	5 per cent	11 "	90± per cent
<i>Puccinia sydowniana</i>	"	22 "	Rare	41 "	2-5 per cent
<i>Puccinia windsoriae</i>	"	10 "	+	41 "	5± per cent

These results demonstrate the effect on germination of continuous wetting (floating on water) of the spores. On examining the tables on germination of *Puccinia ruelliae*, *P. peridermiospora*, *P. windsoriae* and *P. andropogonis* it will be seen that there is little difference between the effect on germination of continuous floating on water and alternate wetting (soaking) and drying. If the period of wetting and drying be added to the incubation period in most cases it is approximately the same as that required for germination when spores are simply floated on water. In other words the effect on germination of the two treatments is essentially the same. The main factors concerned in these treatments are unknown. While the effect of alternate wetting and drying has been known for some time, the writer has found no records in the literature concerning the effect on germination of continuous floating on water.

Teliospores evidently may also change under dry conditions at room temperature. For example only 1 to 5 per cent of spores of *Puccinia peridermiospora* collected March 9, 1920, and tested the next day, germinated in fifteen days. However spores of the same material germinated very well (++++) in 6 days after being kept dry at room temperature until April 8. Moreover spores of *Puccinia andropogonis* collected and

tested as above failed to germinate in 23 days in the first test, but considerable germination occurred in 2 to 4 days in the second one.

As compared with germination in distilled water that in solutions with higher H-ion concentration (pH 4.6 and 5.4) was retarded in the case of *Puccinia asparagi*, *P. sorghi*, *P. ruelliae* and *P. menthae*. On the other hand it was improved for *P. helianthi*. *Puccinia helianthi* will germinate in solutions having a wide range of H ion concentration (approximately pH 3.5–8.4) but the limits for good sporidia production are narrower (approximately pH 4.6–6.5).

#### CONCLUSIONS

Teliospores of certain rusts having a more or less definite rest period may germinate to some extent in December or earlier. Germination is especially favored by prolonged floating on water and by alternate wetting (immersion in water) and drying. As the season advances there is a marked increase in the percentage of spores that will germinate and a decrease in the time necessary for germination to begin, and for complete germination (highest possible percentage of germination).

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## BITTER PIT IN APPLES: THE CRUSHED CELL THEORY.

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McAlpine's five voluminous reports<sup>1</sup> on his investigation of the bitter pit question present a tremendous amount of detailed research into the climatic and other factors operating in the production of the disease and also put forward methods for its control. The actual explanation of the pitting, however, is not convincing, and the Bursting Cell Theory is unsound. In the following account, the crushed Cell Theory is put forward as being the explanation of the trouble. It does not involve the assumption that prior to death the pit cells must swell to the bursting point.

A brief outline of the characteristics of the disease is necessary to stress the main points on which the interpretation of the cause rests.

The presence of sunken dark spots on the surface, resembling hail-marks except that they are scattered over the entire surface instead of only on one side is the first evidence of bitter pit. In the case of the Rome Beauty the diseased areas occur in patches, which sometimes converge around the apple. In this condition the disease is known as confluent pit or crinkle. As McAlpine points out, the pit originates in the vascular network just beneath the skin, the blemishes appearing to a lesser extent deep-seated in the flesh of the apple. In section it is seen that the pits consist of collapsed dead cells *filled with starch*.

The Bursting Cell Theory as summarized by McAlpine<sup>2</sup> is as follows:

When there is an extra rush of sap following on dry conditions, the rapidly swelling pulp cells at the external boundary burst the vascular network at localized spots and the sap pressure which is sufficient to rupture the enveloping network also bursts the thin-walled pulp cells at these particular spots and death of the cells ensues. Briefly, it may be stated that rapid alternations between dry and moist conditions, combined with fluctuating temperatures, during the growing stages of the fruit are the exciting causes of bitter pit.

There are several points in this theory which are not supported by the following facts:

<sup>1</sup> McAlpine, D. Bitter pit investigation. First Progress Rept. 1911-12. 197 p., 33 pl. Literature, p. 111-117. Fifth Progress Rept. 1915-16. 144 p., 37 pl. Literature, p. 67.

<sup>2</sup> McAlpine, D. Bitter pit in apples and pears: latest results in preventive measures. *Phytopath.* 11: 366-370. 1921.

- a. Sunken spots constitute the first external symptoms.
- b. The skin is intact over the pit areas.
- c. The vascular bundles frequently extend through a pit spot and supply normal tissue beyond.
- d. Starch is present in the pit cells.
- e. The tensile strength of cellulose is sufficient to eliminate the possibility of bursting.

These points will be dealt with below but in view of their interdependence they will not be discussed under separate headings.

It is generally accepted that the fluctuation in water supply is the factor primarily responsible for bitter pit. The point in question, however, is in what manner the diseased patches are produced. The rush of sap to the apple after a fall of rain following a dry spell causes a distension of the parenchymatous pulp cells. If the cells in any particular pit area were to distend to such an extent as to cause their bursting the result should be the local production of wart-like bodies before the bursting and subsequent death took place. As a matter of fact, a sinking of the tissue at the affected points constitutes the first external symptoms observed. In the case of Dunn's Seedling (Monroe's Favourite) the flow of sap after a rain following a dry season is sufficient to cause the expected distension of the pulp cells to take place. The force of the distension is sufficient to cause a bursting of the skin. No bursting of the cells takes place within the apple; in fact, it is difficult to see how this could be possible because the expansion of adjacent cells would bring the tissue into a state of static equilibrium and the only relief possible for the increased pressure would be either a bursting of the skin (as in the Dunn's seedling) or a crushing of some of the cells. There is not sufficient internal space in the apple for the expansion of cells to the bursting point. If cells are dissected out and placed in water the amount of water imbibed is not sufficient to cause bursting although under this condition they are freed from the natural obstacle of adjacent turgid cells which is found in the unruptured apple, and which helps to prevent their expansion. An osmotic pressure of 100 atmospheres when entirely borne by the cell may cause bursting but this is enormously beyond anything met with in the cells of the apple.<sup>1</sup> In this case there is the further factor of the pressure from neighboring cells so that bursting of these internal cells is impossible.

The vascular tissue may frequently be traced through a pit and found to be supplying healthy tissue beyond. This would not be the

<sup>1</sup>Dixon, H. H. Transpiration and the ascent of sap. *Progressus Rei Botanicae* 3: 1-66. 7 fig. 1909.

case if the vascular net-work were burst as the Bursting Cell Theory assumes.

The presence of large quantities of starch in the dead cells is another point to be considered. Pitting takes place in the apple for the most part at the time when the starch is being converted into sugar. It is evident that when there is a sudden rush of sap into the apple, the cells which have had their starch converted into sugar will swell to a greater extent and more rapidly than those cells which are still supplied with starch. The rapid distension of the cells is resisted on the outside by the skin. Their force of expansion results in the crushing of those cells whose starch transformation is backward. This accounts for the presence of quantities of starch in the dead pit cells.

It is suggested, though it remains to be proved, that immunity from bitter pit, which is shown in such varieties as Yates, may be due to the uniform transformation of the starch to sugar through the tissue. If such is the case there would be no small clusters of cells far enough behind in their starch transformation to be crushed by neighbouring cells of a higher osmotic pressure.

#### SUMMARY

1. The Bursting Cell Theory does not satisfactorily explain the ultimate cause of bitter pit in apples.
2. The theory is advanced that the affected cells have been killed by being crushed by neighbouring cells having higher osmotic pressure due to their higher proportion of sugar.
3. The presence of starch in quantity in the pit cells, the intact skin over the pitted areas, the first external symptoms being a sinking of the skin, and the fact that a vascular bundle may run through a pit area and supply healthy tissue beyond supports this view.

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## STAINING GERMINATING SPORES

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Investigators have experienced difficulty in making permanent slides showing well differentiated protoplasmic contents of spores, sporidia, promycelia and germ tubes from germinating spores. Lutman (3, p. 1208) transfused germinated spores to microscopic slides bearing albumen fixative, treated the culture with Flemming's weak killing solution and proceeded with the usual staining processes. Osner (4, p. 203) treated his materials similarly but germinated the spores on the albumen fixative. The writer has employed the above methods together with those described by Harper (1) and other workers. The spores either failed to germinate, were lost in the staining and washing processes or lost their turgidity and plasmolyzed so much that the nuclear contents of the germinating parts could not be definitely distinguished. None of the above methods seemed to give assurance that the cultures could be securely retained on the slides for mounting in balsam. This was found especially difficult when iron-alum-haematoxylin stain was used on account of the number and length of washings. Kniep (2, p. 294) employed a method similar to the one herein described and it overcomes many of the difficulties experienced in the manipulations of the other methods. The procedure used by the writer is as follows:

1. Germinate the spores in distilled water, sterilized water or other desirable solutions placed on a slide within a petri dish lined with damp filter paper. Sterilize all apparatus before using and centrifuge the spores in the ordinary way to reduce contaminations.

2. When the proper stage of spore germination has been reached, place five drops of Flemming's weak killing solution on the culture, return it to the petri dish and allow to stand 15 to 30 minutes.

3. Draw the killing solution from the culture by rotating the edge of a circular piece of filter paper held in the margin of the liquid. Wash the culture three times by adding each time several drops of distilled water to the surface and blotting as before. The best results were obtained by allowing the culture to stand for one hour in a fourth washing solution.

4. After blotting off as much water as possible, allow the remaining water to evaporate until the culture has just reached the moist stage. Submerge the slide in water-free ether in a Petri dish and rotate it until all the water is absorbed.

5. Remove the slide from the ether, hold it in a slanting position and pour a two per cent solution of parlodion (or any other good grade of celloidin dissolved in equal parts of absolute alcohol and ether) over the culture beginning with the upper and finishing at the lower end. It is best to pour just enough of the parlodion solution to cover the culture surface of the whole slide and not drain any from the slide.

6. Allow the parlodion to air-dry in a horizontal position until the mottling has nearly disappeared. No difficulty was experienced in proceeding with the next step without drying the parlodion, but when allowed to dry too long, the parlodion film had a tendency to part from the slide. With India ink, label the culture on the parlodion film. However, while the water is evaporating from the culture, the labeling may be placed on one end of the glass slide and, together with the culture, floated with the parlodion.

7. With a pipette, flood, the parlodion film with five changes of alcohol in the following order: 96, 85, 70, 50, 35 per cents and transfer the slide to the stain. The writer secured the best results with the iron-alum-haematoxylin stain. Submerge the slide in a 4 per cent iron-alum mordant.

8. After the culture remains in the mordant for one hour, wash it 5 minutes in running water and then transfer to a 0.5 per cent aqueous solution of Haidenhain's haematoxylin for 2 hours or more.

9. Wash the slide 10 minutes, destain with a solution composed of equal parts of the mordant and water until the parlodion still retains some of the haematoxylin stain. Examination under the microscope will reveal the proper differentiation which was generally found to be at this point:

10. After washing the slide 5 hours, flood the culture with five changes of alcohol in the following order: 35, 50, 70, 85, 96 per cents. Two treatments were employed in the 96 per cent alcohol and the slide well drained. Absolute alcohol should not be used because it dissolves the parlodion and frees the spores.

11. Flood the culture with Eycleshymer's clearing solution composed of equal parts of oil of bergamot, cedar oil and a solution derived from the deliquesced crystals of carbolic acid. Pour off the clearing solution at once and cover with fresh clearing solution which is allowed to remain until the parlodion film is perfectly clear or about thirty minutes.

12. Drain off the clearing solution and mount in balsam.

If the parlodion film parts from the glass slide at the end of any process, the film may be straightened out and floated on the slide under water. If the slide is then drained and ether dropped on the ends of



the parlodion film and more parlodion solution poured over the ends, the culture will remain secure. With careful manipulation every culture can be successfully mounted.

Permanent slides showing well stained germinating spores of the following genera have been secured by this method: *Aspergillus*, *Ascochyta*, *Doassansia*, *Gymnoconia*, *Gymnosporangium*, *Penicillium*, *Phragmidium*, *Puccinia*, *Sordaria*, *Tilletia*, *Uromyces*, *Urocystis*, *Ustilago* and *Venturia*. The iron-alum-haematoxylin stain gave better results than the triple stain. However, the hyaline germ tube walls of the smuts failed to take the haematoxylin stain.

If mounted slides are not stained sufficiently or fade, the balsam may be removed with xylol, the culture treated with the alcohols and restained as previously described. The spore membrane may be made more translucent by treating the culture from 6 to 12 hours in a 2 per cent solution of hydrogen peroxide. The positive assurance that the culture may be retained and the ability to properly stain the materials are the principal merits of this method.

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## NOTE CONCERNING THE DECAY OF WESTERN YELLOW PINE SLASH CAUSED BY *POLYPORUS VOLVATUS* PECK

HENRY SCHMITZ

WITH ONE FIGURE IN THE TEXT

The question of brush disposal in forest operations both in relation to the fire menace and in relation to forest sanitation is one of the most important questions of general forest management in the Inland Empire. In a comprehensive study of the menace involved in neglect of burning slashings after logging operations, Hubert (2) has found that all of the most destructive wood rotting fungi operating in the forests of the north-

west develop fruiting bodies on their hosts after the tree is cut. We are indebted to Weir,<sup>1</sup> working alone and in collaboration with Hubert, for much of the fundamental data dealing with the pathological aspects of forest management in the northwest. Meinecke (3) and Boyce (1) have also made important contributions to our knowledge on this subject.



FIG. 1. Fruiting Body of *Polyporus Volvatus* on a Burnt Yellow Pine Slashing.

In going over the literature, the writer has found only a very few references made to *Polyporus volvatus* Peck in relation to slash or wood decay. Meinecke (4) however, says in part: "*P. volvatus* causes a slow working, rather superficial gray rot, is not considered parasitic; it does not endanger living trees, but is very common." Both Zeller<sup>2</sup> and Schmitz (5) have suggested the possible parasitic nature of this fungus. The present note reports briefly the general occurrence of *Polyporus volvatus* particularly on western yellow pine slash.

During the past several years, the writer has noted fruiting bodies of this fungus on cut western yellow pine logs, saplings and poles, but little attention was paid to the observation. This spring, however, an area several acres in extent has been under observation. This area had a young stand of western yellow pine in the small pole stage. The area

<sup>1</sup> See Hubert, E. E. *loc. cit.* pages 55-56 for a list of these papers.

<sup>2</sup> Unpublished data.

was slashed in the late summer and winter of 1920 and broadcast burned in June, 1921. Although the tops were entirely burned, the bark on the trunks of the trees was only slightly charred. By the spring of 1922, fruiting bodies of *P. volvatus* were common on not only the trunks of trees which had their bark charred but also on the trunks of trees missed by the fire. It is possible that the bark of trees in the neighborhood of the fire, although showing no visible injury may have been devitalized by the heat. Infection in these trees suggests that *P. volvatus* is at least a weak parasite. Fruiting bodies of the fungus were also found nearby on western yellow pine fence posts which were set with their bark still on. These posts are not sufficiently close to the burned area to have been in any way affected by the heat of the fire. Hubert (2) showed that logs in close contact with the ground develop sporophores more readily than logs raised above the ground. Since many of the tree trunks which were considered in the present discussion are not in close contact with the ground, it is problematical how many years sporophores will be produced. Text figure 1 shows the appearance of a fruiting body protruding through the charred bark.

A microscopic examination of the wood revealed the fact that it is in the incipient stages of decay. Fungous mycelium extends generally throughout the wood. Of course, it is a question if this mycelium is that of *Polyporus volvatus*, but since the fruiting bodies of no other fungi were visible, it is not at all unreasonable to believe that it is *P. volvatus*. Experiments are now in progress in this laboratory dealing with the wood destroying proclivities of this fungus.

In conclusion, the writer ventures the opinion that *Polyporus volvatus* may be a much more important fungus than many forest pathologists now suppose.

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## PHYTOPATHOLOGICAL NOTES

*A Fungus destructive to asphalt shingles.*—Views (p. 498) are presented herewith of the two sides of an asphalt shingle heavily overgrown and penetrated by a fungous mycelium. When submitted to the writer some two years ago the statement was made that the reshingling of a roof had been necessitated by the destructive action of this fungus. Later reports indicate that stock in storage is sometimes similarly damaged. The asphalt shingles in question are based on a "felt-paper" similar to that used under carpets. The felt is pulled through liquid asphalt at high temperature and thus saturated and after the excess asphalt is pressed out the shingle is slightly air-cooled and given a coat of natural asphalt, and slate applied on one side. The other side is finished with a mixture of talc and mica for the purpose of destroying the adhesive character of that side of the shingle. The fungus in question is a basidiomycete with mycelium of the general character of that of *Merulius lachrymans*. It grows vigorously, not only over the surface, but it actually penetrates the shingle, subsisting presumably upon the felt paper on which the shingle is based.—F. L. STEVENS.

*The bacterial pathogen of corn stalk rot.*—In view of the rather widespread interest in the bacterial stalk rot of corn, first described from Arkansas (Phytopath. 11: 74–79, 1921), and now reported from about 8 different states, it seems desirable to present a preliminary description of the pathogen. As will be shown in another publication, the organism has not been previously described and for convenience will be briefly characterized at this time.

### ***Pseudomonas dissolvens* n. sp.<sup>1</sup>**

Short, plump, rapidly growing rods, motile by means of a single polar flagellum, bluntly rounded at both ends, occurring usually singly or in pairs, occasionally in short chains, average measurements  $0.7\text{--}1.2 \times 0.5\text{--}0.9\mu$ , capsules present, no irregular forms or spores observed, white on most solid media; colonies on nutrient agar poured plates, testing pH 7.0, round, margins entire, white, opaque, glistening, consistency of melted butter, emitting a strong odor of decaying vegetable matter; gelatin not liquified; acid and gas produced on most nutrient media; diastatic action noticeable; indol and ammonia are produced; Loeffler's blood serum not liquified; nitrates are reduced; milk clears at the end of the fourth day, coagulation delayed but marked on the sixth

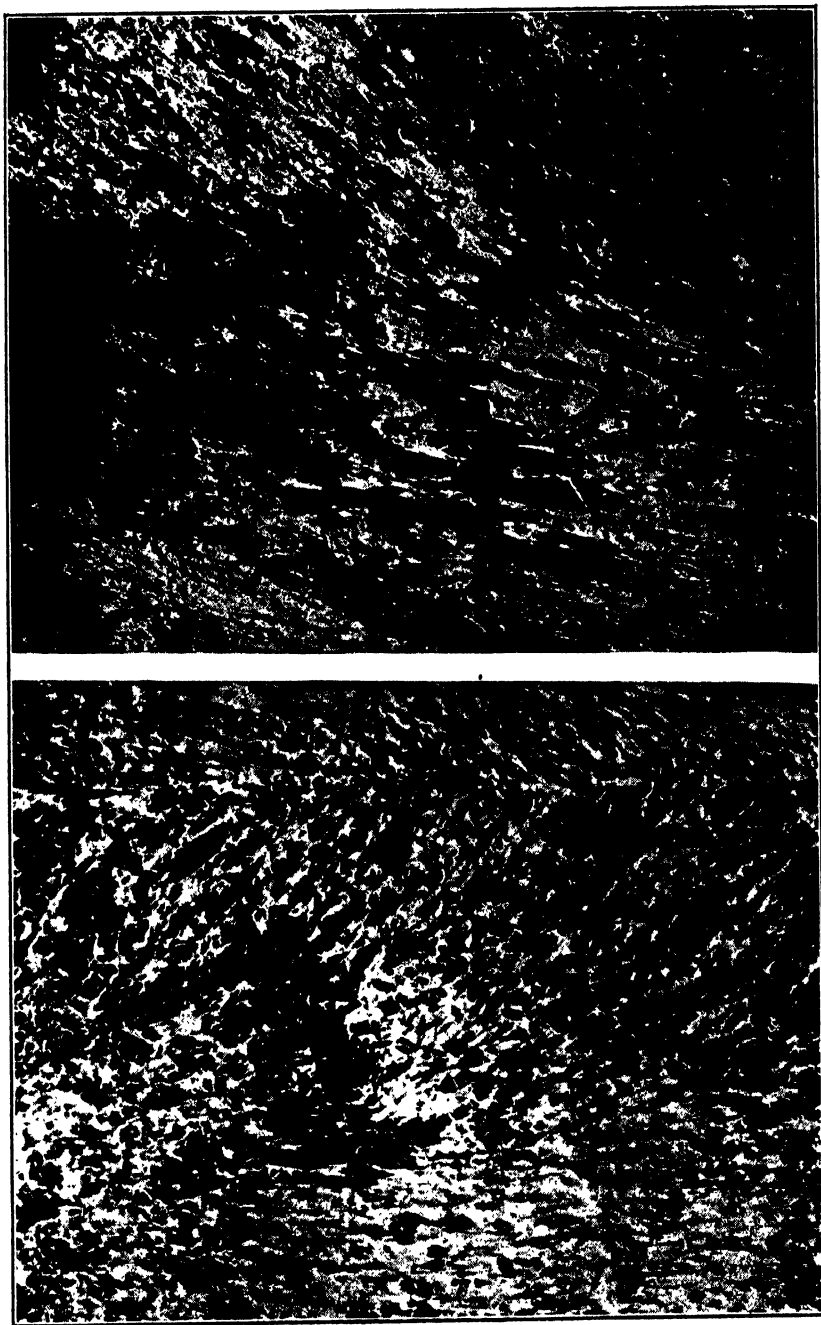


FIG. 1. Photographs showing asphalt shingles penetrated by fungi, viewed from above and below.

day; no growth in Fermi's solution; growth in Uschinsky's solution good. The index number, using the most recent chart endorsed by the Society of American Bacteriologists (1920) is 5322-32220-1111.

Pathogenic on *Zea mays*.—H. R. ROSEN.

*Whetzel resigns headship—deplores present system of administrative tenure.*—Professor H. H. Whetzel who has been for fifteen years head of the Department of Plant Pathology of Cornell University College of Agriculture retired on July 1 from the administrative leadership in order to devote his time and energies more fully to teaching and research together with the immediate preparation of one or more text books. Dr. L. M. Massey who has been acting head for the past year during Professor Whetzel's absence in Bermuda succeeds to the permanent position.

Cornell was the first American university to establish an independent Department of Plant Pathology and has continued as the largest development in its field. When I last visited Cornell a year ago the Department had, in addition to what were doubtless the largest undergraduate classes of any like department in America, an enrollment of twenty-six graduate students. It has during the last thirteen years granted twenty-four Ph. D. degrees with major interest in plant pathology. These men are now scattered in fifteen institutions in the United States. This of itself constitutes a record of service in proportion to opportunity which I can liken, within the range of my personal acquaintance, only with that of the man under whom the undergraduate work of Whetzel and many of the Cornell phytopathologists and botanists was taken—the late Professor M. B. Thomas, of Wabash College. Whetzel has, in all his work, shown a genius for meeting old problems in a new way in the field of phytopathology.

In a letter to his dean setting forth his reasons for wishing to retire from the headship, Whetzel says:

"I am convinced that with rare exceptions no man should be allowed to head a department for more than fifteen years. He should not serve less than ten, unless he is a failure—then the sooner he steps out or is removed, the better. The head of a department who cannot in ten or fifteen years put over a big administrative project deserves no further time or opportunity.

"When a young man I was ambitious, like most of my colleagues, for administrative opportunity and position. Increased salary was, of course, a strong factor, but by no means the strongest. I like the

<sup>1</sup>The name of the organism, using the classification proposed by Smith (Bacteria in Relation to Plant Diseases 1: 171, 1905) is *Bacterium dissolvens* n. sp.

game. I played hard at it. I feel I was in a reasonable measure successful, but my chief interest has always been teaching and research, particularly teaching. Few men can do both administration and teaching, let alone research also with full justice to all. After ten years of administrative work I long to give my time and energies fully to teaching and research work. As you know, I have for four or five years urged you to relieve me of the headship that I might devote myself wholly to my students and my investigations. I feel that another ten years would not only measure the span of my administrative usefulness but would also end any possibility of a successful return to teaching or research.

"One of the most disastrous features of long tenure headship is its effect on ambitious and able young men in the staff. Such men see little hope of administrative opportunity within a reasonable period of time and either leave the institution for a better position elsewhere or become discouraged, discontented, often intriguing and disloyal to the chief. The result in the latter case is sure to be disastrous to some one, usually to the young man himself, often to the department and to the institution, not infrequently to the good name and prestige of the head of the department himself.

"Every university man deserves to opportunity to try for administrative position early in his professional career. The advancement of a man to the headship after he is forty is, in my opinion, rarely warranted. Youth has generally borne the banner of progress in university development as well as in other fields. 'Young men for action, old men for council' and the university may have both, but not to the fullest advantage under our present system of administrative tenure.

"I have had the direction of this department in the College from its beginning. I have seen it expand in fifteen years into one of the largest in the institution. I love it as a father loves his child. I want to see it grow and prosper. I could not bear to see it stagnate or die, as not infrequently happens when one retains administrative responsibility too long. I am sure a younger man can carry it forward farther and with greater success than I could now. Successful administration demands long hours, large personal sacrifices, great energy, and the resiliency of youth. I have given as generously and as completely of these as I could for a decade and a half. I feel that I have earned the privilege of now devoting myself to those phases of my profession nearest my heart. I know that I can teach, I wish to do it. I think that I can do research work, and I wish to try it."—L. R. JONES.

# PHYTOPATHOLOGY

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## THE BACTERIAL SPOT OF PEPPER<sup>1</sup>

B. B. HIGGINS

WITH PLATES XXXI AND XXXII AND FIVE FIGURES IN THE TEXT

A bacterial spot or wart on the leaves, stems, and fruits of peppers (*Capsicum annuum* L.) has been prevalent and quite destructive in the Pimento growing region about Griffin, Georgia, during the last three seasons. It was first brought to my attention during the spring of 1920, when some 2000 Pimento pepper plants were bought from a local grower, for field work in relation to a study of pepper fruit rots. The plants were examined carefully for signs of disease before being set in the field. A few were thrown out because of small brownish spots on the leaves. These spots were distinctly different from the common *Cercospora* leaf spot, especially in the absence of the white center so characteristic of the *Cercospora* spot.

The remainder of the plants were set in the field about the middle of May, together with several rows of other healthy plants. Six weeks later every plant in the lot purchased from the local grower was diseased and the disease had spread to most of the other plants in the field. Many of the diseased leaves had already dropped off. During August many of the plants were almost completely defoliated.

### SYMPTOMS

The spots on the leaves, generally, first appear as small, circular, pale green pimples. They are raised on the under surface of the leaf and occasionally on both surfaces. Usually there is a slight depression or concavity on the upper surface. On old leaves the first noticeable sign of infection is often a darker green, water-soaked spot. The behavior and final appearance of the spots vary considerably with the age and growth conditions of the leaves attacked. Usually the center of the spot dies and collapses after a few days. The spot continues to enlarge

<sup>1</sup> Paper Number 17, Journal Series, Georgia Agricultural Experiment Station.



for some time, forming a circular or oblong pale-yellow or straw-colored spot 1 to 10 mm. in diameter with a border of water-soaked tissue which finally turns dark brown. On old leaves, late in the fall, the spots are dark brown with a paler brown center on the lower surface. Occasionally two or more spots coalesce and form a larger spot of a more irregular outline. However, large areas of the leaf are never killed. Usually, when infection is abundant, the spots are small and do not coalesce, the entire leaf turning yellow and dropping off, or quite as often dropping off while still green.

On the stems the disease is not very noticeable. Small, elongated, raised cankers 3 to 5 by 1 to 2 mm. in diameter appear and go through much the same series of transitions as the spots on the leaves. The center of the cankers finally becomes roughened and light brown in color; but the spots do not collapse as they do on the leaves.

On the fruits the spots are much more conspicuous. They are circular in outline, 2 to 5 mm. in diameter, raised with a cracked and roughened surface, (Pl. XXXII) giving them a wart-like appearance. They are at first pale green but soon turn brown. During damp weather decay organisms often enter through these spots and cause the collapse of the surrounding tissue and finally of the entire fruit.

#### CAUSE OF THE DISEASE

Soon after the disease was noticed in the fields, isolations were made by dilution cultures on agar plates. The leaves were washed, immersed for one minute in a 1-1000 mercuric chloride solution, and then washed in sterile water. The diseased spots were then removed with flamed tweezers and crushed in tubes of melted agar from which other dilution poured plates were made. When young spots were washed and cultured in this manner pure cultures of a yellow-pigment-forming bacterium, which reproduced the disease when sprayed onto pepper plants, were obtained.

#### MORPHOLOGY OF THE ORGANISM.

*Vegetative cells.* These bacteria were found to be short rods with rounded ends. When grown on beef extract agar and stained with gentian violet or methylene blue the cells measured 1 to 1.8 by 0.5 to .7  $\mu$ . They usually occur singly, but occasionally in pairs and rarely in short chains. The nature of the substratum seemed to affect the size and shape of the bacterial cells only slightly. In carbohydrate media there seemed to be a slightly increased tendency to form chains.

*Capsules.* Capsules are present and quite distinct on certain media. They have been demonstrated in 24 hour old beef extract agar cultures,

but they are very much more distinct in old cultures on sweet potato plugs (Fig. 1).

*Endospores.* No evidence of endospores could be obtained by staining. Temperature reactions of old cultures indicate that they are not formed.

*Motility.* The organism is not very actively motile. Hanging drop mounts from more than a dozen young cultures were examined before motility was satisfactorily demonstrated. Usually only a very small percentage of the cells are motile, though occasionally a larger number

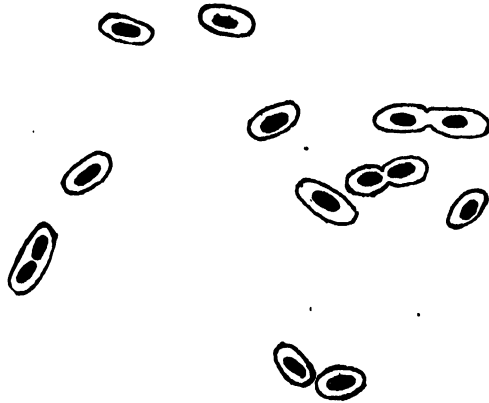


Fig. 1. Rods with capsules from a 20-day old sweet potato culture, stained by the Welch method.  $\times 3700$ .



Fig. 2. Monoflagellate rods from a 20-hour old agar culture stained by Loeffler's method.  $\times 3200$ .

were found active. Flagella stains demonstrated the same thing. That is, flagella could be found on only a few cells; although they stained readily with Loeffler's methylene blue or with carbol-fuchsin when preceded by a mordant. It has only a single polar flagellum which is usually about two and a half times the length of the cell (Fig. 2).

*Involution Forms.* No involution forms of any kind were found. In staining for capsules by the Welch method many irregular forms were found, but these were evidently due to plasmolysis from the salt solution

used in washing. When mounts from the same cultures were stained with gentian violet or with methylene blue the cells appeared entirely normal.

*Staining.* The organism takes the routine bacteriological stains readily; but it is Gram negative. Mounts from eighteen and twenty-four hour beef extract agar cultures were tested on several occasions, but with Gram's stain the results were always negative.

#### CULTURAL CHARACTERISTICS

All cultural and physiological studies were made with at least four isolations of the organism as follows: 1, from a leaf spot in 1920; 2, from a leaf spot in 1921; 3, from a fruit spot; and 4, from the hypocotyl of a young seedling. In addition, the reaction of several other isolations were from time to time compared with these four.

The organism is a moderately rapid grower. On poured plates of beef extract agar incubated at 25° C. the colonies are visible to the unaided eye after about 40 hours. In plate cultures it is easily separated from the saprophytic forms, commonly occurring in or on the diseased spots of the host plant, by means of the color and general appearance of the colonies. By reflected light the surface colonies are some shade of lemon yellow from the time they appear until they dry up. They are wet, shiny, and slightly convex. By transmitted light they are translucent, slightly opalescent, and the larger colonies show a distinctly translucent border around the more opaque center. Until about the fifth day the edges of the colonies are smooth. In older plates they become reticulate or lobate (Pl. XXXI, fig. 1). The submerged colonies are lens shaped, but often break through and spread out on the surface. In old cultures they usually become more or less irregularly lobed (Pl. XXXI, fig. 2). The colonies between the agar and the glass plate are thin, delicate, and of a pale cream yellow color.

The growth in agar stroke cultures is filiform at first but after a few days it becomes more or less echinulate. In agar stab cultures the surface growth is not abundant, not covering the surface of the agar. The submerged growth is echinate.

*Gelatin.* On beef extract gelatin the color of the bacterial growth is approximately the same as that produced on agar. In thinly sown plates the colonies are 1 to 1.5 cm. in diameter, after 10 day incubation at 18° C., with a cup-shaped liquefied area underneath. In stab cultures some growth occurs all along the stab, although no liquefaction is discernible except along the upper portion. In stab cultures in freshly prepared gelatin liquefaction is evident at the upper end of the stab after 48-hours growth at 20° C. A cup shaped or turnip-shaped area

0.5 cm. deep is liquefied. By the fourth day this area has become more crateriform. By the seventh day the entire upper portion of the gelatin is liquefied to the depth of 1 cm. or more, and below this a flat Lima bean-shaped area is liquefied with the bacteria settled into a sickle-shaped mass at the bottom. In older gelatin which has been heated several times the liquefaction is more rapid but much less characteristic.

*Bouillon.* In beef extract broth the growth seems to occur almost entirely on the surface, and, if allowed to stand undisturbed, the liquid is not very decidedly clouded. When shaken, the growth becomes distributed, producing a decided cloudiness, but gradually settles to the bottom. In cultures 3 weeks or more old the liquid is clear and more translucent than sterile broth, the bacterial growth having formed a viscid mass at the bottom. The entire liquid gradually becomes lemon yellow and mucilaginous. The bacterial growth at the bottom is straw yellow and has about the consistency of egg albumen.

*Potato.* On steamed Irish potato plugs growth was a little more abundant than on beef extract agar, slightly viscid, of a dense cream yellow color, but lacking the opalescence of agar cultures. Where the plugs were very moist the growth was of a more fluid nature, and had a tendency to spread and flow to the bottom of the tube. There was very little digestion of the potato tissue.

*Sweet potato.* On steamed sweet potato plugs growth is very rapid and abundant, decidedly more abundant than on any other vegetable medium tried. By the end of 10 days at a temperature of 20° to 25° C. the plugs are almost engulfed in a yellow, viscid mass which flows to the bottom of the tube. By the end of a month the plug is almost entirely digested. Usually a bluish-green color develops in the sweet potato tissue by the fifth day. This color did not develop in plugs from sweet potatoes taken from a curing house late in the spring. This variation was probably due to changes in the latex of the potatoes.

*Pepper fruit agar.* Pepper fruit agar was prepared in two ways. By the first method 400 grams of green Pimento pods were minced fine and boiled for an hour in a small amount of tap water. The liquid was then drained off, filtered, and made up to 1500 cc. To this liquid 1.5 per cent agar was added, steamed until dissolved, filtered, tubed, and sterilized. By the second method only the sap of the pepper was used. The Pimento pods (400 grams) were ground in a food chopper and the sap (300 cc.) pressed out, filtered, and then made up to 1500 cc. by the addition of tap water. Agar was added as above.

The freshly expressed sap had a hydrogen-ion concentration of approximately pH 5.2. The acidity was slightly increased by sterili-

zation. The agar prepared by the first method was a little more acid, having a pH value of 5.0.

The sap made much the clearer, nicer agar. When slants of either were heavily inoculated, some growth was produced, but when a small amount of inoculum was employed no growth resulted. However, after neutralization by the addition of a normal sodium hydroxide solution, growth was very good—much more abundant than on beef extract agar.

*Egg albumen.* Good growth was produced in a 2 per cent solution of commercial dried egg albumen, with or without the addition of 0.5 gram of  $\text{KH}_2\text{PO}_4$  per liter.

*Asparagin.* No growth was produced in asparagin solution.

*Media lacking organic nitrogen.* No growth was produced in a solution of inorganic salts plus saccharose ( $\text{K}_2\text{HPO}_4$ , 0.5 gram;  $\text{CaCl}_2$ , 0.5 gram;  $\text{KNO}_3$ , 1.0 gram; saccharose, 10.0 grams; and water, 1000.0 cc.). It seemed that some organic nitrogen was required, though the amount needed was not large. Good growth was obtained on a 0.1 per cent solution of peptone, especially when dextrose or saccharose was added. Increasing the proteid content to the amount recommended by the Committee (1) on the Descriptive Chart of the Society of American Bacteriologists by the addition of beef extract and peptone (0.3 per cent beef extract and 0.5 per cent peptone) did not noticeably increase the growth.

*Carbohydrate media.* The organism responds very quickly to the addition of dextrose or saccharose to beef extract agar or broth. On stab cultures in tubes of beef extract agar the growth never covers the surface of the agar. When 3 per cent of either of the above sugars are added a solid mass of bacterial growth 1 to 1.5 cm. deep is produced above the agar. Lactose, maltose, mannite, glycerine, or starch did not increase the growth by any such appreciable amount, although some of them, at least lactose and starch, are used to some extent.

#### PHYSIOLOGY

*Liquefaction of gelatin.* In gelatin stab cultures incubated at 18° to 20° C. liquefaction begins on the second day and proceeds slowly until growth ceases which is about the end of the sixth week. If the gelatin is more than two inches deep in the tube, it is usually not all liquefied.

*Relation to free oxygen.* The organism is a strict aerobe. No growth is produced in the closed arm of fermentation tubes containing beef extract broth and various dissolved carbohydrates. Some growth is produced along the needle puncture in agar and gelatin stab cultures. In agar shake cultures no colonies develop in the depths of the media.

Numerous small colonies develop at the surface but none can be seen with a hand lens at a depth of 2 mm. or more.

*Effect of light.* In thickly sown plates of beef extract agar the bacteria were all killed by a 75 minute exposure to bright sun-light. In neutral pepper sap agar plates only about two-thirds of the organisms were killed, the darker color of the agar protecting them to some extent.

*Acid production.* In beef extract broth or agar containing 1 per cent dextrose, saccharose, maltose, or glycerine no evidence of acid production could be obtained. After the second day the medium in every case became gradually more alkaline. Following the observation that some alkaline substance was formed in the cultures, efforts were made to avoid the obscuring effect of the alkali on the production of acids. The first method tried was to increase the sugar content and use agar instead of broth. Three per cent dextrose, saccharose, and lactose were added to beef extract agars. Brom thymol blue was then added as an indicator and sodium hydroxide solution was added until the media were neutral (grass green). The agar was then tubed and autoclaved 20 minutes under 10 pounds pressure. After a preliminary incubation, the tubes of agar were inoculated and the cultures incubated at 25° C. After 48 to 72 hours the agar about the bacterial growth had changed to the full alkaline color of the indicator. By the sixth day the color had returned to nearly neutral in the tubes containing dextrose and saccharose. By the eighth day the agar immediately around the bacterial growth had become decidedly acid yellow. This color gradually passed down through the agar until, by the end of a month, it was yellow throughout. The agar with lactose became deeper blue and the color never changed to yellow.

Acid production was not indicated when the organism was grown on beef extract broth plus 3 per cent of these sugars with the indicator added. The difference was probably due to the slower diffusion of the proteids through the agar.

By reducing the protein content of the broth, and consequently the production of alkali, it was found that very appreciable quantities of acid could be demonstrated with certain sugars. A one tenth per cent peptone solution plus 3 per cent dextrose or other sugar and brom thymol blue as indicator served beautifully for this purpose. In all cases there was a slight increase in alkalinity during the second and third days. By the fifth day the color had changed to yellow in the tubes containing dextrose or saccharose, and by the end of 3 weeks the reaction was orange to methyl red. With lactose the growth was not so rapid. Apparently the lactose was not used so long as any other nutrient was present, since no indication of acid production could be seen until the end of 2

weeks. After that the acidity increased rapidly, and the final reaction was similar to that obtained with dextrose and saccharose. With maltose the growth was fairly good but no acid was produced at the end of 6 weeks. With mannite or glycerine growth was very scanty. Apparently the organism was not able to use these substances.

*Ammonia production.* Ammonia was produced consistently in all media tested. In beef extract broth, in Dunham's solution, and in solutions of egg albumen the presence of ammonia was shown repeatedly by browning of filter paper wet with Nesler's solution and placed in the mouth of the culture flask or test tube. Furthermore, quantitative tests indicate that all the alkali formed is volatile and appears to be ammonia (gives brown color with Nesler's solution).

TABLE 1

*True acidity and change in reaction of beef extract broth after six weeks incubation at 25° C.*

Acidity (Fuller's)		NaCl series				Beef extract broth series					
		neutral	+5	+10	+15	+11	+12	+13	+14	+15	+16
Check	pH	8.5	7.4	5.6	4.8	6.4	6.3	5.8	5.4	5.1	5.0
	cc. N/20 NaOH to neutralize 10 cc.					1.35	1.7	2.0	2.2	2.4	2.6
Cultures, average of 4 flasks	pH	8.7	8.8	8.4	8.4	8.4	8.3	8.3	8.4	8.4	8.3
	cc. N/20 NaOH to neutralize 10 cc.					0.45	0.50	0.55	0.65	0.70	.087

*Reaction of medium.* The organism grows well in broth neutral to phenolphthalein and in broth with a moderately acid or moderately alkaline reaction. In one series, in which 1 per cent NaCl was added to the beef extract broth, growth was obtained in neutral, +5, and +10, — 5, — 10, — 15, and — 20 (Fuller's scale) but not in + 15 or — 25. Growth started first and appeared to be best in neutral and + 5. No growth was apparent in the — 15 until about the tenth day or in the — 20 until near the end of 3 weeks. In another series without the addition of NaCl growth was obtained in +10, + 11, + 12, + 13, + 14, + 15, and + 16, — 11, — 12, — 13, — 14, — 15, — 16, — 17, and — 18. In the — 18 evidence of growth was not visible, but at the end of 30 days loop transfers to agar plates showed a large number of living

bacteria while in + 17 and - 19 or above the organisms were dead. It will be noted that growth occurred in the + 16 in this case whereas it did not occur in + 15 in the NaCl broth series. This is explained by the fact that the true acidity was greater in the latter case as shown in table 1.

*Indol production.* Eight tubes of Dunham's solution were inoculated and with two sterile tubes to serve as checks, were incubated at 25° C. At the end of 6 days all were tested for indol by adding at once 1 cc. of a 0.02 per cent NaNO<sub>2</sub> solution and 1 cc. of concentrated sulphuric acid. In all of the inoculated tubes a red color began to appear within a few minutes and gradually darkened to a brick red, while no red color appeared in either of the check tubes. At various times, also, positive results have been obtained with the vanillin test in cultures in Dunham's solution and in other media.

*Phenol production.* No indication of phenol production was obtained in any medium.

*Reduction of nitrates.* Beef extract agar plus 1 per cent KNO<sub>3</sub> did not show the presence of nitrite when tested on the second, fifth, and tenth days. Beef extract broth plus 1 per cent KNO<sub>3</sub> and 2 per cent saccharose gave positive tests on the sixth day. No growth could be obtained on nitrate media lacking organic nitrogen.

*Color reduction.* In litmus milk the color was at first destroyed but began to reappear at the surface of the liquid after 2 weeks. In litmus broth a similar behavior occurred. In broth tinted with methylene blue the color change was very striking. The color disappeared entirely from the depths of the liquid by the end of the fifth day, while it still remained intense in the upper centimeter. By the end of 10 days it had disappeared entirely, but it soon reappeared beginning at the surface of the liquid. In the beef extract agar plus 3 per cent saccharose and tinted with methylene blue the color disappeared throughout the depths of the agar by the sixth day. A thin film remained over the surface of the agar, even covering the bacterial mass.

*Digestion of egg albumen.* Ten grams of egg albumen were dissolved in 500 cc. of tap water. Fifty cc. of the solution were then placed in each of 10 flasks and autoclaved under 10 pounds pressure for 30 minutes. The albumen was coagulated into rather large lumps. Eight of the flasks were inoculated and, with the 2 checks, were incubated at 25° C. By the sixth day some evidence of digestion was visible; and by the end of 6 weeks practically all of the albumen had been dissolved, leaving a fairly clear yellowish liquid.



## INOCULATIONS

Hundreds of plants have been successfully inoculated; but details will be given in only a few cases, which will serve to give a general idea of the methods used.

On July 16 an 8-day old bouillon culture was diluted with an equal volume of sterile water and sprayed with an atomizer onto seven young Pimento pepper plants. At the same time 7 similar plants were sprayed with sterile water as checks. All were covered with bell glasses for 72 hours. On the tenth day spots began to appear on all inoculated plants, at first as pale green or whitish pimples which collapsed and turned brown after a few days. By the fifteenth day infection was abundant. Forty-seven spots were counted on one leaf. All of the check plants remained healthy.

The organism was reisolated from one of the inoculated plants. Diseased tissue was cut out, dipped into strong alcohol, soaked 2 minutes in a 1-1000 solution of mercuric chloride, washed in sterile water, and then crushed in a tube of melted agar. Dilution cultures from this tube gave an abundance of the yellow colonies of the original organism with practically no contamination.

On September 22, 2 Tabasco pepper plants, one bell (Royal King), and 3 Chili pepper plants were sprayed with a water suspension of bacteria from a 4-day old agar culture isolated from spots on a bell pepper fruit. These plants with a like number of checks were then covered with bell glasses for 48 hours. On the eleventh day small whitish pimples began to appear on the under surface of the leaves on all inoculated plants. On the Tabasco leaves the spots were small (about 1 mm. in diameter). The spots did not enlarge to any appreciable extent later and the leaves were not distorted.

On March 14, 3 tomato (Globe) plants and two bell (Royal King) pepper plants were sprayed with a water suspension of bacteria from a 2-day old agar culture of the organism reisolated from a pepper leaf inoculated 2 months previously with bacteria from a pepper fruit spot. These 5 plants and a similar number of checks were covered 48 hours with bell glasses. On the tenth day infection was evident on the leaves and stems of the pepper plants and on the leaves of the tomato plants. A month later 155 spots were counted on a single leaflet from tomato and 299 on a somewhat larger pepper leaf. The spots on the tomato leaves were small (0.5 to 2 mm. in diameter), light straw yellow to dark brown in color, and raised on the lower surface or with a sunken center and slightly raised border. On pepper leaves the spots were very similar, but averaged slightly larger than on the tomato. Raised warty streaks, 1 mm. wide by 3 to 5 mm. long were produced on the pepper stems.

The organism was reisolated from spots on both pepper and tomato. The checks remained healthy.

All attempts to infect tomato fruits have given negative results.

A few infections have been obtained on both Pimento and Chili pepper fruits; the percentage of infections however, was very small. In the field natural infection was very abundant on fruits set during damp weather in the early summer of 1921. During the dryer weather of late summer it was difficult to find an infected fruit, although, at the time, infection was very abundant on the leaves. Observations indicate that there is only a short period in the development of the fruit when it is susceptible to infection and that is probably during the development of the lenticels on the young fruits soon after the petals fall. One difficulty in the way of studying artificial inoculation in young fruits is the fact that they drop off, if kept under very moist conditions.

When the bacteria are pricked into the flesh of green pepper fruits, they usually grow and kill the surrounding tissues; but the typical warty fruit spot is never produced.

*Incubation period.* The incubation period at ordinary summer temperatures is about ten to fifteen days. Signs of infection are usually visible on the younger leaves on the tenth day and they continue to appear until about the fifteenth day. A temperature of about 15° C. increased the incubation period one third. Above 20° C. temperature does not appear to be the limiting factor. The acidity of the pepper sap doubtless accounts for the slow development of the spots. The acidity of the sap of the pepper plant, at least of the fruit, is very near the limit for growth of the bacteria.

#### PATHOLOGICAL HISTOLOGY

*Materials used.* For the early stages of infection bacterial suspensions were placed in droplets on the under surface of pepper leaves. The inoculated plants were then covered with bell glasses for 72 hours. On the third, seventh, eleventh, and twelfth days after inoculation the inoculated spots were cut out and killed in Durand's modification of Gilson's fluid or in a mixture of equal parts 95 per cent alcohol and glacial acetic acid. The killed material was then dehydrated, imbedded in paraffin, sectioned, and stained with various stains. The best staining was obtained with Haidenhain's iron-alum haematoxylin.

For the later stages spots in various stages of development on both leaves and fruits were killed, imbedded, sectioned and stained as for the early stages.

*Bacteria within the host tissue.* In material for the study of the very early stages of infection the bacteria were apparently lost in the killing

and dehydrating processes, so that the exact mode of penetration was not observed. In material killed on the eleventh and twelfth days many early stages of spot formation were found.

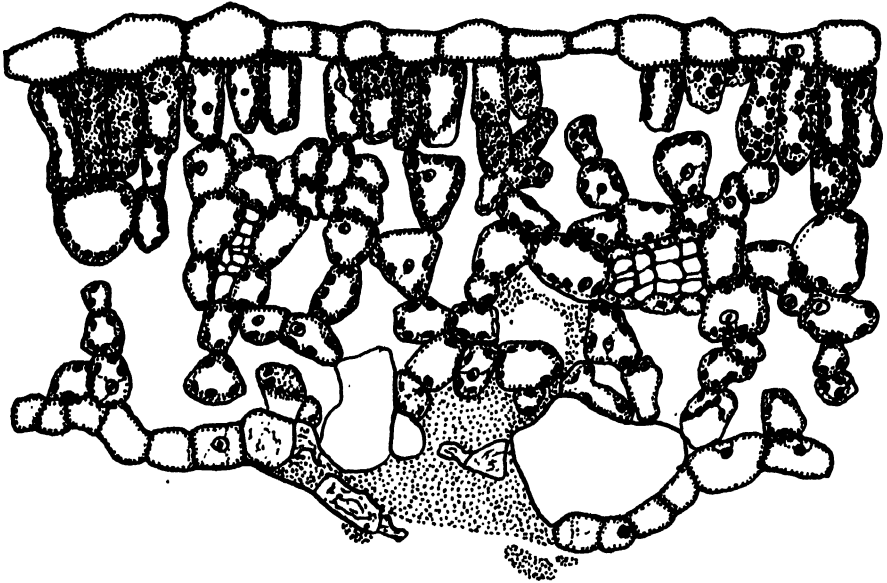


Fig. 3 Section of pepper leaf spot, showing an early stage of spot formation. The sub-stomatal cavity is filled with slimy bacterial growth (heavily stippled). The swelling of two mesophyll cells has broken the epidermis.

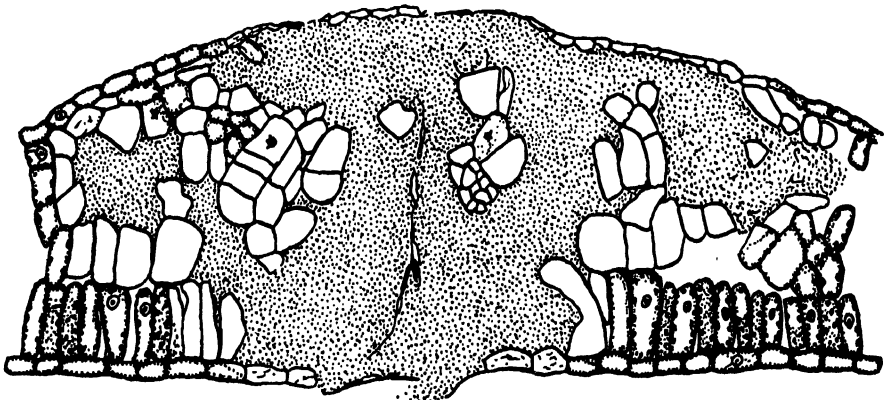


Fig. 4. Section of a leaf spot at a later stage than that shown in figure 3.

At this time slimy masses of bacteria were found within the stomatal cavity and in the adjoining intercellular spaces (Fig. 3). The cells in contact with this mass swell and break the epidermis, producing the pimples mentioned under "Inoculations." From this point the bacteria

spread to the intercellular spaces throughout the thickness of the leaf (Fig. 4), forcing the cells apart and finally killing and crushing them. After this the spot dries and the tissues collapse, the bacteria continuing to spread around the edges of the spot for some time thereafter (Fig. 5A).

The bacteria are imbedded in a mass of slimy material and especially in old spots, the individual bacteria can rarely be distinguished. This material seems to be hygroscopic and is doubtless responsible for a part of the swelling at certain stages; but a great part of the swelling is due to the actual enlargement of the host cells (Figs. 2, 5 A, 5 B). In young tissues in both leaves and fruits there is some stimulation to cell division. The hypertrophy is probably due to stimulation by small amounts of ammonia given off by the bacteria as suggested by Smith (7) in his discussion of crown gall. The final death of the cells may be due to excessive stimulation by the ammonia or to the coagulation and final digestion of the cell proteids by the bacteria. As the cells begin to swell the chloroplasts disappear. Later the cell membrane and the nucleus disappear. The nucleolus often persists after the cell is dead.

Early stages in the development of the fruit spots were not studied. In the later stages developments are very similar to those found in the leaf spots. The surface tissues collapse and become cracked and roughened (Fig. 5 B). The bacteria continue to advance laterally and toward the interior between the host cells. In old spots they often penetrate entirely through the flesh and into the seed cavity.

#### IDENTITY OF THE CAUSAL ORGANISM

The first mention of a bacterial spot of peppers, which I have been able to find, is in a short note on a leaf spot by Heald and Wolf (5) in their Plant Disease Survey of Texas. In 1918 Sherbakoff (6) gave quite a complete description of the disease as it occurs on the leaves and fruits of pepper in Florida. Recently a very similar disease of tomatoes was studied by Miss Doidge in South Africa. This disease was found to be caused by a bacterium for which Miss Doidge (2) suggested the name *Bacterium vesicatorium* n. sp. Shortly afterward a more complete description (3) of the disease and of the causal organism was published. Later Gardner and Hendrick (4) described a similar disease of tomatoes in Indiana. Although they refer to the work of Miss Doidge, and suggest that she has studied the same disease, they suggest another name, *Bacterium exitiosum* n. sp., for the causal organism.

The descriptions of the organism as given by Miss Doidge and by Gardner and Hendrick (4) are very similar, although the physiological reactions differ in several important points. The same has been found true in comparing the pepper organism with their descriptions. Doubt-

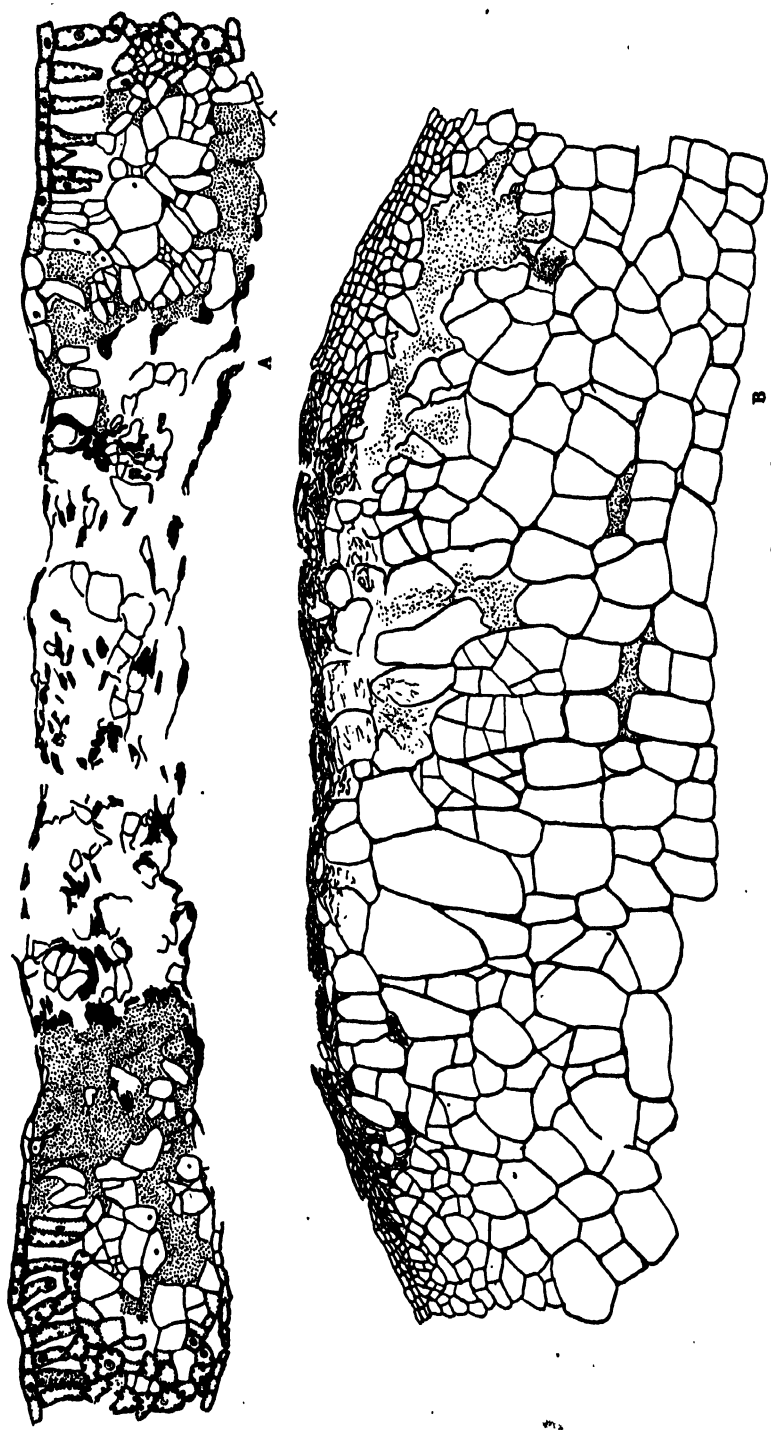


Fig. 5. A. Section of an old spot on pepper leaf. The bacteria are still growing in the border of the spot. Section through a unit spot, showing the collapsed surface tissues and the bacterial masses which are forcing the cells apart in places.

less many, if not all, of the differences are due to variations in technique. Until a comparative study of the three organisms can be made, it seems useless, therefore, to suggest the identity of the pepper organism. According to my own results the group number should be *B. 211. 2222523*.

#### CONTROL MEASURES

The appearance of the disease in seed-beds where peppers had never been grown before was almost conclusive evidence that the bacteria had been brought in on the seed, and led to a closer study of this question.

Commercial pepper seed (Royal King variety) were germinated in sterile soil in the green house, and the seedlings were watched for appearance of the disease. Two lots of 300 seed each were soaked in water 6 hours. One lot was then treated 2 minutes with a 1-1000 solution of mercuric chloride, washed with sterile water, and planted in a large pot of sterile soil. The other lot was planted, without treatment in a similar pot of sterile soil. Of the treated seed 155 germinated from which all but 12 seedlings were healthy. Of the untreated lot 176 seeds germinated from which 126 seedlings developed spots on the cotyledons or on the hypocotyls. The greater part of these spots were produced by *Colletotrichum*, *Gloeosporium*, *Macrosporium*, and other fungi. The pepper spot bacteria were isolated from a number of the spots on the cotyledons and from one spot on a hypocotyl. Two such isolations were used in a number of successful inoculation experiments and in some of the physiological studies. Of the 12 spots on the seedlings from the treated seed 8 were produced by *Macrosporium* and the other four by *Colletotrichum*.

The mercuric chloride treatment was quite effective in control of the leaf spot, but in other tests the germination of the seed was severely injured. Therefore, more extensive studies of seed treatment must be made before definite recommendations can be published. Indications are, that if the disease can be kept out of the seed-bed, it is not likely to be troublesome in the field.

Spraying four times with Bordeaux mixture after the disease had become established in the field, reduced the disease considerably, but did not give complete control.

#### SUMMARY

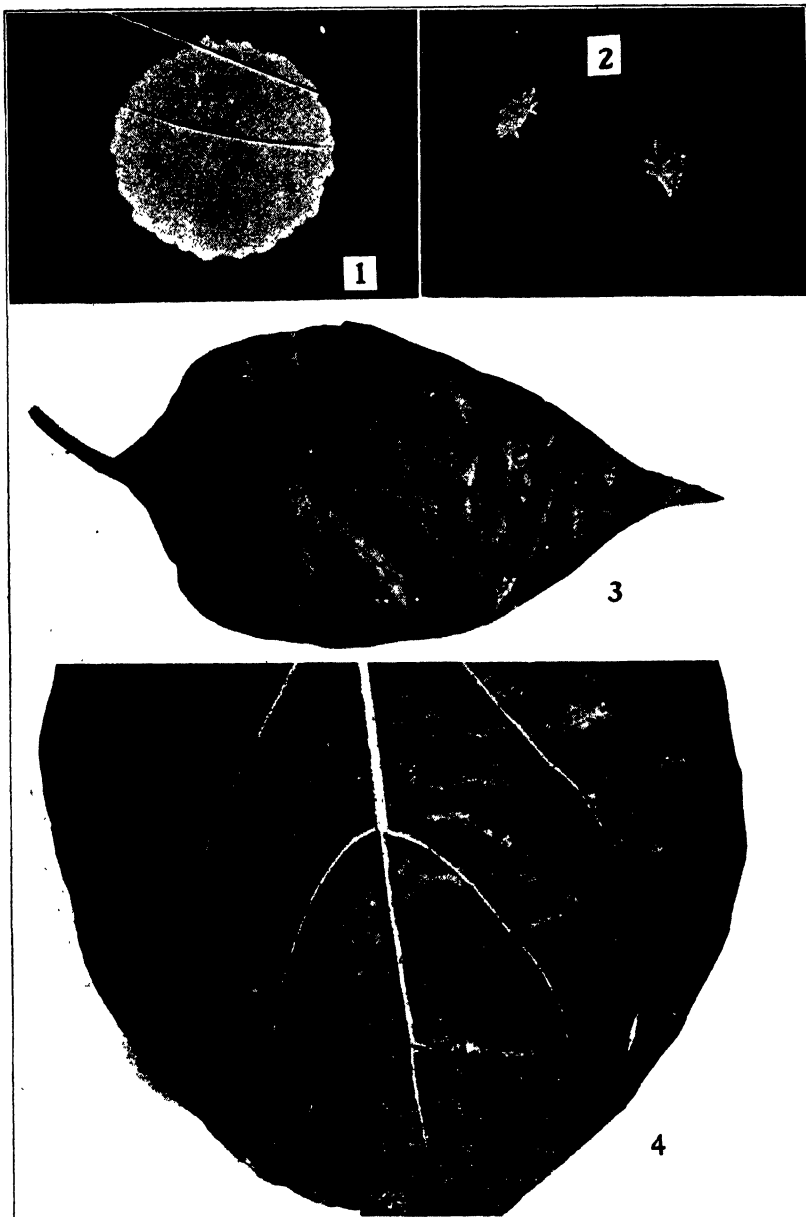
1. The bacterial spot of pepper has been present in destructive form during the last three seasons in the Pimento growing region of Georgia.
2. The disease and the causal organism are described.
3. The disease is caused by a species of *Bacterium* very similar to *B. vesicatorium* Doidge and to *B. exitiosum* Gardner and Kendrick, but

it differs from the descriptions of these forms in some important physiological reactions.

4. The identity of the organism is still uncertain.
5. The causal bacteria are carried on the pepper seed.
6. Seed treatment with a 1-1000 solution of mercuric chloride is effective in controlling the disease, but it often injures the germination of the pepper seed.
7. Spraying four times with Bordeaux mixture gave only partial control.

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BACTERIAL SPOT OF PEPPER

Fig. 1. Surface colony of pepper leaf spot bacteria in 27-day old agar plate culture  $\times 3$ .

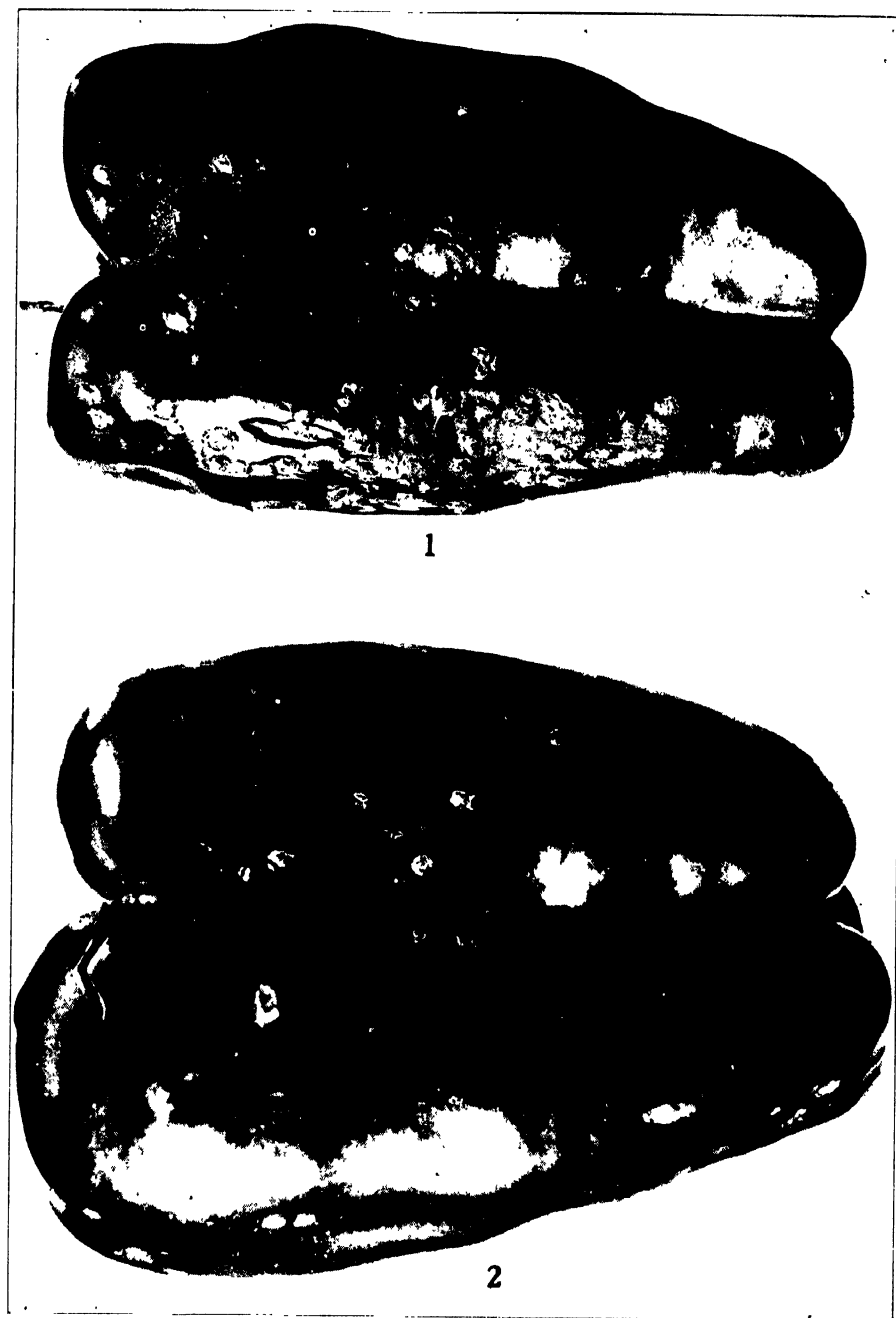
Fig. 2. Two submerged colonies from same culture.  $\times 3$ .

Fig. 3. Bacterial spots on pepper leaf in early summer, showing light brown spots with darker borders.

Fig. 4. Bacterial spots on pepper leaf collected in the field November 11. Note that spots are dark brown. Natural size.







BACTERIAL SROTS ON TWO FRUITS OF BELL (ROYAL KING) PEPPER. Natural size.



## A NEW HOST FOR THE FIRE BLIGHT ORGANISM, BACILLUS AMYLOVORUS

LAETITIA M. SNOW

The first printed notice of fire blight was a report taken from a letter by William Denning, dated December 22, 1793, and published in the Transactions of the New York Society for the Promotion of Agricultural Arts and Manufactures (16). In this letter Mr. Denning stated that he had noticed for some years a "disorder" in apple orchards in the vicinity of the Hudson River above the Highlands, and that he had found it also attacking pear and quince trees.<sup>1</sup> Henry Ward Beecher in 1844 (7) quoted J. H. James and C. W. Elliott as reporting "blight" in various trees and shrubs, such as apple, pear, peach, quince, English hawthorn, privet, black birch, Spanish chestnut, elder and Calycanthus, although he himself had observed it only on fruit trees. In 1846 Downing (17) reported that he had seen it on several other trees besides the pear, for example the Ailanthus, Catalpa, and Spanish chestnut. Barry, in 1847 (6) added crab, walnut, and hickory to the list, and in the same year Eaton (18) suspected the Tartarian cherry of having the disease. Two years later (1849) James (23) noticed the same effect on common cherries and white currants besides several of the trees already mentioned. All the observations above noted were without convincing evidence as to the nature of the malady. In 1870 however, Hull (21) compared the disease in the pear and apple and proved that the "fungus is identical, by inoculation," which was the first experimental evidence offered.

In 1880 T. J. Burrill proved that the disease was due to bacteria, and reported the fact before the Illinois State Horticultural Society. The report in the Transactions of the Society (8) is, according to his own account the first published reference to the causal organism of pear blight and, it may also be added, to the bacterial infection of plants. His experiments included pear, apple, and quince. In the same year (9) he stated that "probably many other plants, among which we now name the butternut, Lombardy poplar, and American aspen, suffer from the same disease." He thought at first (11) that peach yellows was due to the fire blight bacterium, but later concluded (13) that the organisms were not "specifically identical." The butternut disease was charact-

<sup>1</sup> This reference is almost always given in pear blight literature and, with one exception, I have found it incorrectly cited from a Massachusetts publication.

erized, without proof, (10) as similar in every way to sun scald of apple, which he in turn identified with pear blight (12, 14). In one report (11) he stated that the Lombardy poplar was also destroyed by "these ferment producing agents," and that the aspen (*Populus tremuloides*) was similarly affected with blackening of limbs similar to pear and apple blight. Again no proof is offered of the identity of infecting organisms. In the following year, 1882, (13) he considered the Lombardy poplar disease "in cause and consequence" as "assuredly the same thing" as pear blight, stating that it had been communicated from one to the other by inoculations, but giving no details. "The aspen, the maples, the elms, the willows, the mountain ash, the lilac (leaves), the butternut, some herbaceous plants (peony, lettuce, potato?), etc., more or less suffer in the same way on account of the same, or an indistinguishable, voracious, little, but sufficiently potent, parasite." However, he gave no description of the disease, the culture reactions of the organisms, or of cross inoculations. From the above one would conclude that, while Burrill recognized the fact that certain plants were affected in a similar way by bacteria, he offered no, or insufficient evidence as to the identity of the attacking organisms.

Arthur in 1885 (2) reported the observation of the disease on "hawthorn," and successful inoculation experiments (4, p. 357) on service berry, English hawthorn (*Crataegus oxyacantha*), and an evergreen thorn (*Crataegus pyracantha*). A third article in the same year (3) added an observation of the disease on crab, confirmed Burrill's statement regarding its presence on mountain ash, and reported unsuccessful attempts to inoculate grape, raspberry, mulberry, peach, etc. A fourth article in 1885, (3, p. 375) stated that the effect of the fire blight organism upon peach was not the characteristic blight, but gummosis. Inoculations into black raspberry and green grapes yielded no results. Later (1907) Ralph E. Smith (30) attacked "peach blight" from another angle and concluded that it was not caused by the fire blight organism but by a fungus. In 1887 Arthur reported successful inoculation experiments on wild crab (*Pyrus coronaria*) but was unable to produce the disease on elderberry, abele poplar, or balm of Gilead.

In 1894 Sturgis (32) reported twig blight on Spaulding variety of plum, but was unsuccessful in obtaining either pure cultures of the organism or infection in apple, pear, or quince trees. He added, as the opinion of Arthur, that it was probably not identical with pear blight. In 1902 Jones (25) proved, by culture reactions and pathogenicity, that the pear blight organism was the cause of the blight of the Cheney plum (*Prunus americana nigra*), and quoted F. A. Waugh as reporting its presence in the Hortulana group also. In the following year Paddock

(28) proved by cross inoculations and culture reactions, that the apricot blight was caused by the same organism. He also thought that a blight on *Prunus simonii* was due to the same form, but offered no proof. Although Craig, (15) had reported in 1897 the presence of the disease on English hawthorn in Ottawa, in 1907 Edwards (19) stated that in Ontario neither English hawthorn nor any of the wild species of *Crataegus* had the disease. He found, however, that a double scarlet variety (*Crataegus oxyacantha* var. *splendens*) was subject to it, as was also a cut-leaved mountain ash (*Pyrus aucuparia* var. *laciniata*). In the same year (1907) Waite (33) added two plants to the list of "susceptibles," *Eriobotrya japonica* and *Heteromeles arbutifolia*. Whetzel and Stewart in 1909 (34) after listing the common hosts for the disease added "a few ornamentals," and stated that the form "appeared" to be on prunes. This was confirmed later (1915) by Jackson (22) by means of inoculations. O'Gara (27) in 1910 stated that loquat started one case of fire blight, but gave no evidence. In 1913 Hewitt (20), in his account of the history of the disease, stated that it was reported by various authors on pear, apple, quince, crab apple, peach, almond, plum, haw (several species of *Crataegus*), mountain ash, service berry, red raspberry, and black berry.<sup>1</sup> Reed in 1914 (29) specified among the "haws" *Crataegus crus-galli* as being specially susceptible to blossom blight. Munn in 1918 (26) was able to produce fire blight in strawberry blossoms by means of spray inoculation, but stated that it had not been found to occur in nature.

#### SUMMARY AS TO REPORTED HOSTS.

As the matter now stands, it has been proved, either by inoculations alone or in connection with culture reactions for the purpose of identification, that the fire blight organism is able to infect the following hosts: pear, apple, quince, service berry (*Amelanchier canadensis*), English hawthorn (*Crataegus oxyacantha*), evergreen thorn (*Crataegus pyracantha*), wild crab (*Pyrus coronaria*), cultivated crab, Cheney plum (*Prunus americana nigra*), apricot, prune, and strawberry. The claim for the Lombardy poplar is questioned. Other reports are presumably from observational evidence only, as they are given without any record of inoculation or the isolation and identification of the causal organism.

In the following report a new host, *Prunus triloba* var. *plena*, is added to the list.

<sup>1</sup> In regard to several of these hosts the writer has not been able to find any other reference.

## ISOLATION

While working in the Department of Plant Pathology of the University of Wisconsin in the summer of 1919, my attention was directed by Professor L. R. Jones, to a twig blight of the ornamental shrub *Prunus triloba* var. *plena* in several localities near the University. A number of cultures were isolated from a clump of shrubs of this variety growing on Observatory Hill, and were labelled "B", but at this time no successful isolations were obtained from other specimens. Later (July 1920) a strain was isolated from a twig taken from a specimen growing in the garden of Professor Jones, and sent to Wellesley by Mrs. R. C. Williamson. This strain was labelled "J." Several strains were isolated at the same time as "B" from crab apple, Northwestern Greening apple, and from a species of hawthorn sent to the laboratory. The strain from the crab ("A") proved to be especially virulent, and was used, with a laboratory stock culture ("Wis. 165") for a control in the subsequent work. In the following descriptions the four strains (A, B, J, 165) will be understood to show like characters unless otherwise stated.

## MORPHOLOGY AND CULTURE REACTIONS

*Morphology.*—The organism isolated from flowering plum is a short, motile, non-spore bearing rod, varying in size ( $J = 0.5$  to  $0.7 \mu$  by  $1$  to  $1.9 \mu$ ,  $B = 0.5$  to  $0.7 \mu$  to  $1.9 \mu$ ) and apparently becoming shorter with age. Shunk's method of staining revealed 1 to 2 flagella, chiefly at the ends, although occasionally one appeared to be attached at the side. The flagella were most abundant in A, rare in B, and fairly abundant in J and 165. All the strains were Gram negative (Sterling's method). No capsules were observed.

*Agar streak.*—Greyish white, J inclined to be brownish when old. Opacity varying with amount of growth; growth more vigorous on potato agar than on nutrient agar and inclined to be somewhat darker in color. Streak slightly depressed in the center, edge clearly defined; smooth and shiny. Growth of B and J somewhat more abundant than A and 165.

*Agar colonies.*—Round, finely granular to the edge, which is entire at first but may be wavy later; shiny, smooth, usually slightly raised. If deep, may be round or lenticular. Colonies of 165 were not noted.

*Gelatin stab.*—White in A, 165 and B; slightly yellowish or brownish in J. Growth best at top. Liquefaction so slight at the end of 30 days as to be questionable; not discernible in J. This result was obtained in 1921, and possibly may be correlated with the loss of peptonizing power to be noted below. Arthur (1) reported no liquefaction in two weeks, but other writers record slight liquefaction.

*Litmus milk*.—In 1919, immediately after isolation, A and B showed a faintly acid reaction after 3 days, becoming neutral after a week, with a soft coagulum, and a little peptonization at the top. After 2 weeks the soft curd was alkaline and peptonization had proceeded one third to one half way down the tubes.

In 1921 the action was very slow; 165 showed no change after 12 days while the others had started to change. No acid was evident. All the cultures became slightly alkaline and somewhat faded at the bottom. B and J were a little more alkaline than A and 165. No coagulation was noted after 12 days, but by 30 days a soft coagulum was present, least firm in 165. No peptonization occurred. This change in reaction must have been due to some common cause, for it occurred in A as well as in B, and in as much as the 1921 reactions of A and B agreed with those of J and 165, which were not tested in 1919, long cultivation is suggested as the causal factor.

*Sugar broths*.—No gas was produced at any time. In each case the culture was cloudy in the outer part of Dunham's fermentation tube, and clear in the inner tube. There was little sediment and a slight pellicle (a mere ring in J). The total acidity was tested by titration with the following results.:

Dextrose	+7.0 to +13.5	Fuller's scale (165, A, B, J)
Maltose	+2.0 to + 6.0	" " (A, B, J)
Lactose	—7.0 to + 1.0	" " (A, B, J)
Sucrose	+6.8 to + 7.5	" " (A, B)

*Nitrate reduction*.—The experiment lasted nearly 4 weeks and the tubes were tested after 4, 9, and 27 days. No nitrogen was evolved and no nitrites were formed, but a moderate amount of ammonia was produced, the most by B. Jones (24) and Stewart (31) state that no ammonia is formed. These experiments were repeated several times with the same result and the writer feels certain that nitrates are reduced to ammonia by strains A, B, J, and 165, but without nitrites or nitrogen being produced.

*Indol and hydrogen sulphide*.—In Dunham's solution, after 2 weeks, no hydrogen sulphide or indol were produced. These results agree with those of Jones (25) as regards hydrogen sulphide, but differ with respect to indol. Jones (24) and Stewart (31), however, report no indol formation.

*Potato*.—For this work plugs were cut from raw potatoes, placed in tubes and sterilized. The first two sets were sterilized by the discontinuous method, and the third in the autoclave. The method of sterilization appeared to be unimportant. Three series were run, one in 1919



and two in 1922. The first two showed considerable variation both in the cultures of the same strain and in different strains. In the third set there was some slight variation between the different cultures of the strains, but the different strains showed almost absolutely identical variations. In the last series B was, on the whole, somewhat lighter in color and more abundant than the others. At first all were dirty white, becoming somewhat darker with age, J tending toward a brownish tint and the other three toward yellow. In all, the growth was scanty to fairly abundant, smooth and shiny at the start, becoming drier with age. In the first series A had a distinctly sour odor, which was not noted in B, (165 and J were not included in this series). In the second series the same odor was noticed in A and 165, while that of B and J was very faint and difficult to characterize. This peculiar, faint odor was characteristic of all the cultures in the third set. I cannot account for this variation unless it was due to the age of the cultures used in transferring. In the third set all cultures were selected as good, vigorous ones of the same age. Many authors have noted a characteristic odor (variously described) in blighted plants. Arthur (5) calls it a weak, peculiar odor, not like decay. Others speak of it as "sour." The growth on potato agrees more nearly with that described by Arthur (1) than with that described by other authorities.

#### PATHOGENICITY

Immediately after isolation B was pathogenic for pear (green fruits) but inoculation into fairly young shoots of the flowering plum yielded no results. The fact that this was done about the middle of the summer may account for the negative results, but it has not been possible thus far to make inoculations in the early spring. After a year's cultivation one culture of this strain was very slightly pathogenic for pear. This sub-strain was lost during experimentation, and the work was continued with a culture, which when tested in 1920, proved to be non-virulent. A had remained strongly virulent after 2 years of cultivation. J was only weakly pathogenic for pear when isolated and lost virulence almost entirely after a year's cultivation. Wis. 165 was not found to be virulent when obtained from the laboratory.

#### SUMMARY

1. Strains B and J, isolated from blighted twigs of *Prunus triloba* var. *plena*, were found to be pathogenic for green pear fruits with characteristic blighting and formation of milky exudate.

2. Compared with strain A (isolated from crab apple) and 165 (from the laboratory of Plant Pathology, University of Wisconsin) the culture

reactions of B and J agree. They also agree, in the main, with reactions given by other investigators. The following exceptions may be noted:

(a) In the formation of ammonia from nitrate all four strains differ from previous reports.

(b) During cultivation the power to digest casein was lost, with a corresponding slight or no liquefaction of gelatin.

(c) No indol was formed by any of the strains.

(d) The color of the growth on potato differs from that reported by several investigators, but agrees with that given by Arthur.

3. During cultivation 165, B and J lost their virulence while A remained strongly pathogenic for pear.

I wish to extend my thanks to Professor L. R. Jones, Department of Plant Pathology, University of Wisconsin, for personal suggestions and helpful criticisms and for the departmental courtesy extended to me during the progress of the investigation. My thanks are also due Professor Franz Aust of the Department of Horticulture for the identification of the host plant.

WELLESLEY COLLEGE,  
WELLESLEY, MASS.

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# YEAST-SPOT OF LIMA BEANS<sup>1</sup>

S. A. WINGARD

WITH FOUR FIGURES IN THE TEXT

Examination in the fall of 1921 of diseased Lima beans, *Phaseolus lunatus* L., from eastern and central Virginia revealed the presence of the vegetative cells, asci and ascospores of a yeast which was constantly associated with the affected tissue. The organism has been readily isolated from several lots of beans by the following method: The affected seed were immersed for about a half minute in 95 per cent alcohol, some of the granular tissue removed from the lesion on a sterile needle and placed in a tube of sterile water. After shaking vigorously to break up the clumps of ascospores, several drops of the suspension were transferred to petri dishes, which were then poured with beer wort agar. These platings invariably produced numerous yeast colonies free of all contamination.

The yeast under discussion clearly falls under the genus *Nematospora* which was described by Peglion<sup>2</sup> in 1901. There are two species described in this genus, both of which are plant pathogens namely *Nematospora coryli* Peg. described from hazel nuts in Italy by Peglion in 1901, and *Nematospora lycopersici* Sch. described from tomato fruit by Schneider<sup>3</sup> in 1916. The form on Lima beans differs from these in certain characters and is apparently an undescribed species. The name *Nematospora phaseoli* is therefore proposed. A description of it is appended.

## DIAGNOSIS

### ***Nematospora phaseoli* n. sp.**

*Form.* The cells show a wide variation in form. The elliptical and spherical types as shown in figure 1, A predominate in young cultures. Other forms are found in varying numbers depending upon the conditions under which the cultures are grown. Mycelium-like strands, and cells

<sup>1</sup> Paper 59 from the Department of Plant Pathology, Virginia Agricultural Experiment Station. Read at the Toronto Meeting of the American Phytopathological Society, December, 1921. See abstract in *Phytopathology* 12: 47. 1922.

<sup>2</sup> Peglion, Vittorio. Über die *Nematospora coryli* Pegl. *Centralbl. f. Bakt. Abt. 2*, 7: 754-761. 1 pl. 1901.

<sup>3</sup> Schneider, Albert. A parasitic saccharomycete of tomato. *Phytopathology* 6: 395-399, 4 fig. 1916. Further note on a parasitic saccharomycete of tomato, *Phytopathology* 7: 52-53. 1917.

in the shape of tennis rackets, walking sticks, etc., are not uncommon in 24 hour cultures.

**Arrangement.** The organisms commonly occur in groups. In young cultures 1 to 5 buds are usually attached to the mother cell as shown in figure 1, A. In some cases the mother cell sends out a group of bud cells from one end and a mycelium-like strand from the other. These strands are septate and, in most cases, form buds at the crosswalls.

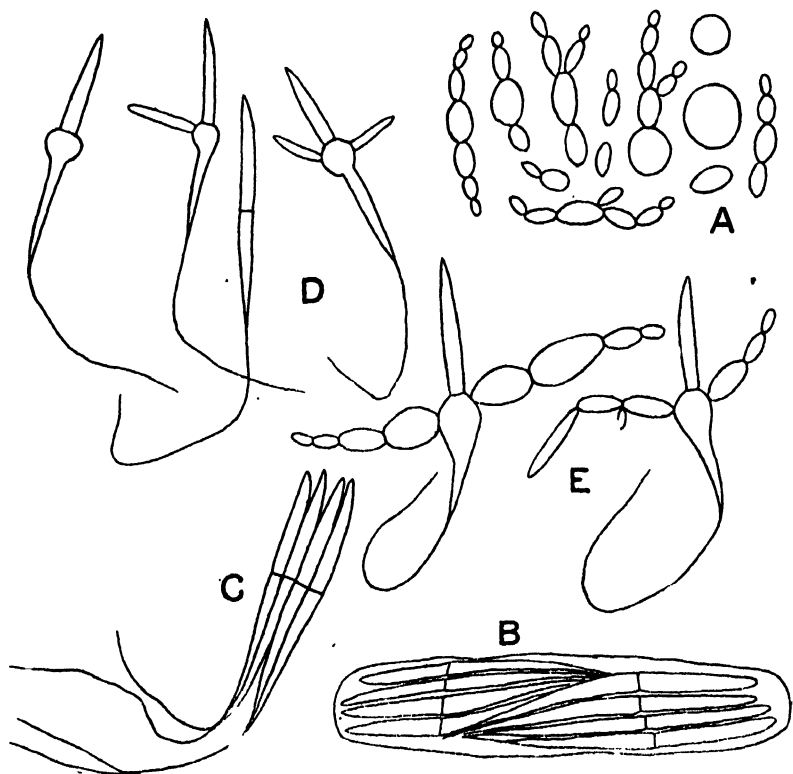


Fig. 1. *Nematospora phaseoli*. A, Vegetative cells, showing the characteristic budding; B, mature ascus, showing the arrangement of the ascospores; C, a cluster of four ascospores clinging together after being released from the ascus; D, germinating ascospores, showing the mode of germination in which a typical germ tube is produced; E, germinating ascospores, showing the budding mode of germination.

**Size.** Elliptical cells in young cultures vary in length from 8 to 14  $\mu$  and in diameter from 6 to 10  $\mu$ . The mature spherical cells are 20  $\mu$  in diameter, and the mycelium-like strands vary in length from 90 to 140  $\mu$  and in diameter from 2.5 to 3.5  $\mu$ .

**Asci and ascospores.** Asci and ascospores are produced in great numbers in the lesions on Lima bean seed and also on favorable nutrient media. The asci are cylindrical with rounded ends (Fig. 1, B and Fig.

2, C), 60–85  $\mu$   $\times$  10–12  $\mu$ ; ascospores 8, in two groups of 4, 40–46  $\mu$   $\times$  2.5–3  $\mu$ , slender, 1-septate, slightly ridged at septum, apex acute, base extended into a slender, non-motile whip, which averages about one and one-fourth times the spore length (Fig. 1, D, and Fig. 2, D).

*Ascospore germination.* Two modes of spore germination have been observed. In the first and most common type, the basal cell swells at the transverse septum forming a sphere about 6  $\mu$  in diameter from which a germ tube protrudes and grows into a mycelium-like strand of considerable length, with crosswalls and branches, which give it the appearance of a true mycelium (Fig. 1, D). In the second the sphere is formed as in the preceding but instead of sending out a septate strand, spherical cells (Fig. 1, E) bud off from it.

*Staining Reactions.* The vegetative cells, asci and ascospores stain readily with the ordinary stains; the reaction to the Gram stain is positive.

#### CULTURAL CHARACTERISTICS

No extensive study has been made of the growth of the organism on different culture media, but it has been grown on several media to determine which are most favorable for its development. In general it grows best on media that are most favorable for the growth of the ordinary yeast, that is, on beer wort agar, and on fresh vegetable material such as garden beets, Irish potatoes and sweet potatoes.

*Beer wort agar.* On beer wort agar plates at a temperature of 30° C., the colonies appear on the second day. They are convex, circular, with entire margins and smooth surface, opaque, dull. The internal structure is finely granular (Fig. 2, B). The colony is cream colored at first but gradually turns brown with age. By the end of the seventh day the colony has attained its maximum size, which is from 5 to 10 mm. in diameter (Fig. 2, A). Asci and ascospores are produced in great numbers in 48 to 72 hours from the time of plating. After about three weeks, the colony becomes surrounded by a border of mycelial growth. A second crop of asci is produced on these mycelial branches.

On beer wort agar slants there is an abundant growth, which is slightly filiform in appearance, raised, dull, cream colored, opaque, contoured, with a butyrous consistency (Fig. 2, G). An alcoholic odor is apparent.

*Whey agar.* The organism made a good growth on one lot of this medium, but failed to grow on other lots which were prepared later.

*Corn meal.* The yeast develops poorly on corn meal agar, but grows well on steamed corn meal and water.

*Potato and beet.* Fresh garden beets, Irish potatoes, and sweet potatoes have proved to be very favorable media (Fig. 2, E and F). In some cases

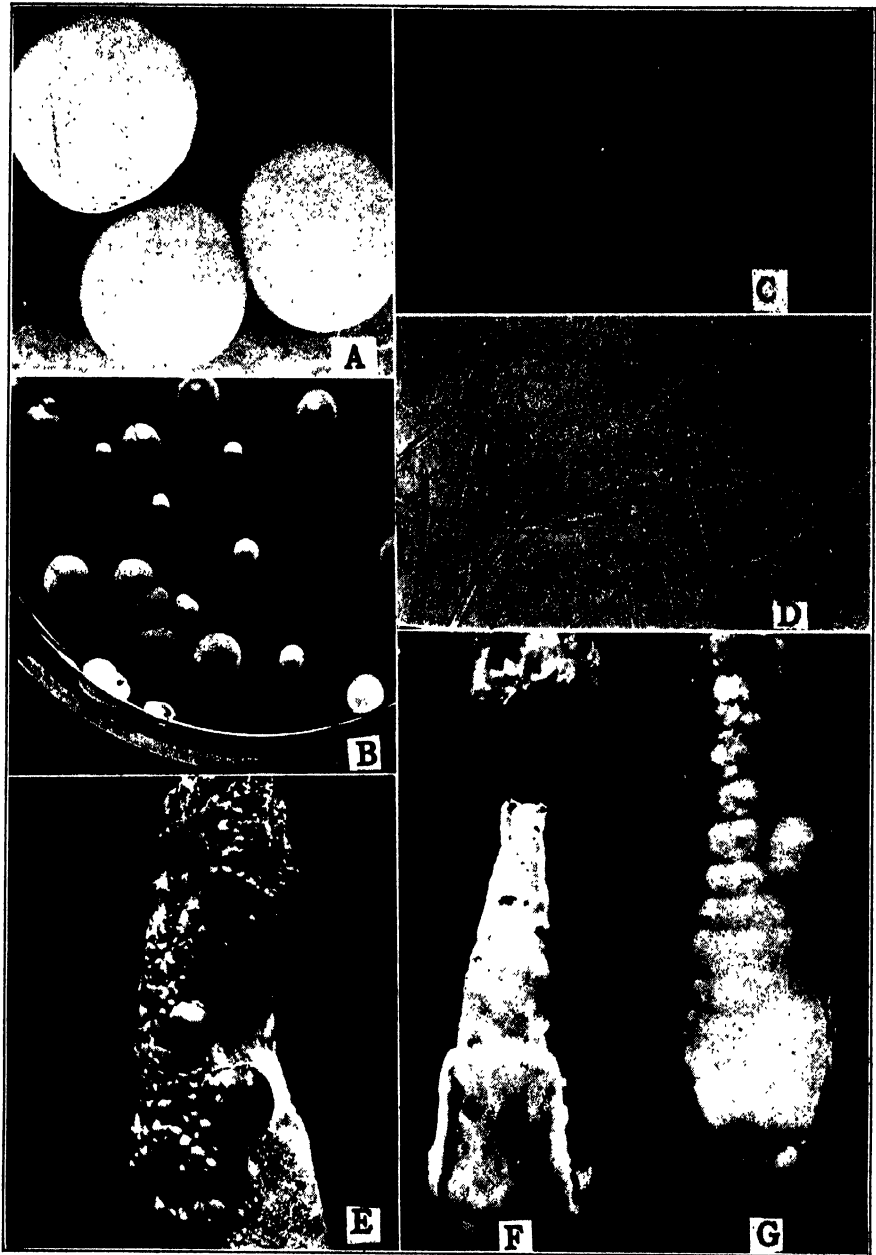


Fig. 2. *Nematospora phaseoli*. A, Eight-day growth on beer wort agar. 2.5  $\times$ . B, Ten-day growth on beer wort agar at 30° C., about natural size. C, Vegetative cells, asci and ascospores, about 200  $\times$ ; D, Mature ascospores, about 400  $\times$ . E, One-month growth on Irish potato, 2.5  $\times$ . F, Eight-day growth on sweet potato, 1.2  $\times$ . G, Eight-day growth on beer wort agar, 1.2  $\times$ .

the slants are completely covered by the growth of the yeast in 48 hours from the time of inoculation.

*Beef peptone agar.* This is a very poor medium for the growth of the organism. Colonies are produced but they never attain a diameter of more than 1 to 2 mm. Asci and ascospores occur very sparingly in these colonies.

*Czapek agar.* No growth is made on this medium.

#### TEMPERATURE RELATIONS

The optimum temperature for growth has not been carefully determined but it appears to be about 30° C. The organism makes a very poor growth and forms asci and ascospores very slowly at a temperature of 18° C.

#### APPEARANCE OF THE DISEASE

The disease occurs on the seed in the pod. It causes numerous dark sunken areas on the cotyledons. The organism is apparently able to attack the seed at any time during development, but the most severe injury seems to result when infection takes place before the seed is half grown. In case of early infection the seed may either die prematurely or fail to grow to normal size (Fig. 3, A). Affected seed vary from one-tenth to natural size. In the majority of cases the testa remains unbroken, the infected spot being dark brown and somewhat sunken and wrinkled (Fig. 3, C). This, however, is not always true, since in some cases the testa is ruptured the disease manifesting itself in the form of crater-like lesions on the cotyledons (Fig. 3, B). The tissue in the lesions is grayish-brown in color, and granular in texture. In the lesions are found great masses of ascospores, and a small number of vegetative cells and young asci.

The disease has only been observed on the seed up to the present time. Pods that appear perfectly healthy may contain badly diseased seed.

#### DISTRIBUTION

The original specimen of this disease was collected in York County, Virginia, October 15, 1921, by Mr. A. G. Smith, Jr.<sup>1</sup> Additional collections were made by Mr. Smith in this and six other counties in the State. Specimens of the disease have been collected from the following counties: York, Henrico, King and Queen, King William, Essex, Albemarle and Dinwiddie. The ease with which the specimens were found indicates that the disease is rather prevalent and widely distributed.

<sup>1</sup> The writer is greatly indebted to Mr. A. G. Smith, Jr., Specialist in Vegetable Gardening, Virginia Extension Division, for collecting affected material in the field



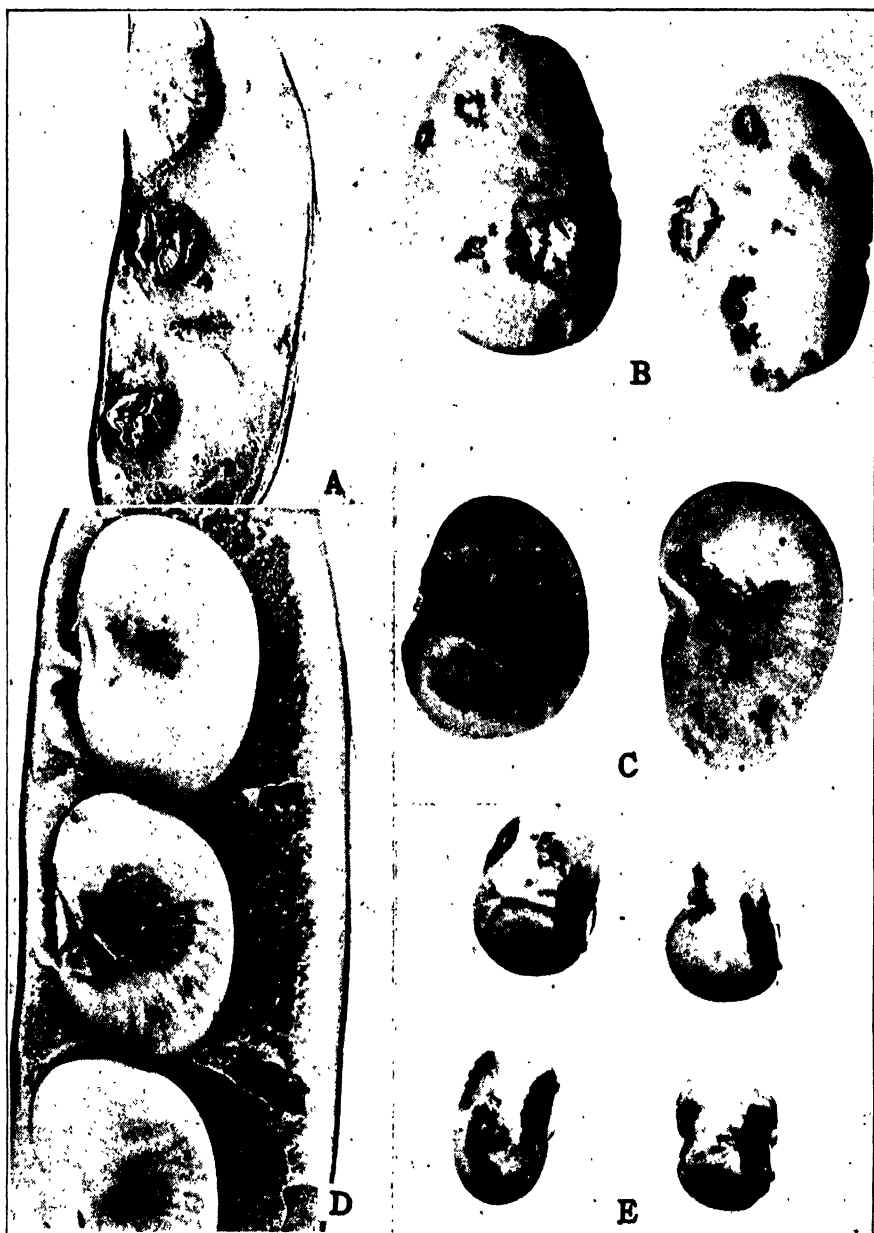


Fig. 3 *Nematospora phaseoli*. A, Lima bean pod and seed showing the result of natural infection in the field, 1.5  $\times$ . B, Natural infection showing crater-like lesions and ruptured testa, 3  $\times$ . C, Natural infection on Lima bean seed, resulting in dark brown sunken spots on the cotyledons in which the testa remains intact, 3  $\times$ . D, Artificial infection on Lima bean seed, 2.5  $\times$ . E, Yeast-spot infection on Blackeye cowpea seed, 3  $\times$ .

The percentage of infection varied from a trace to as much as 60 per cent. of the crop.

Affected seed of several different varieties of Lima beans have been collected, but the small Lima or sieva type seems to be especially susceptible to the disease. One specimen of blackeye, cowpea has been collected which was affected with the same or a very similar yeast (Fig. 3, E).

#### PATHOGENICITY

The pathogenicity of the yeast on Lima beans has been demonstrated repeatedly in the greenhouse. Spraying the young pods by means of

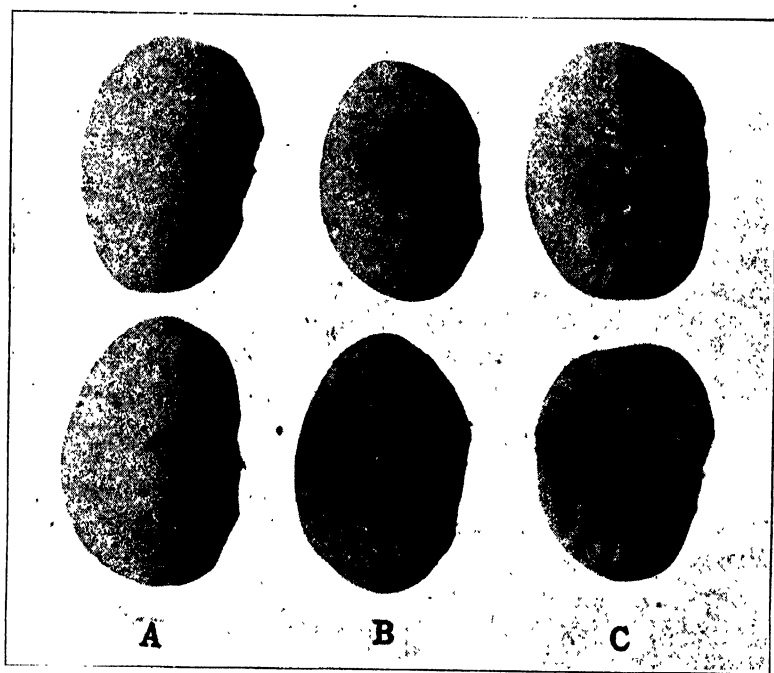


Fig. 4. *Nematospora phaseoli*. A, Two Lima bean seed which were used as controls in a greenhouse inoculation experiment, 2.5  $\times$ . B, Two Lima bean seed which were aerificially inoculated in the greenhouse, 2.5  $\times$ . C, Two Lima bean seed showing natural infection in the field, 2.5  $\times$ .

an atomizer with a water suspension of a pure culture of the organism failed to produce infection. Negative results were also obtained when the pods were smeared with a pure culture of the yeast grown on agar. The seed was readily infected when the pods were punctured with a needle, dipped into a pure culture of the yeast. Additional inoculations of the seed made in a similar manner resulted in infections closely resembling those produced under natural conditions (Fig. 3, D and

Fig. 4, B). The characteristic symptoms are evident within two or three days after inoculation. They are quite conspicuous within 7 to 10 days.

No infection was produced on garden beans, *Phaseolus vulgaris*, in the greenhouse. Tomato fruits in different stages of development were inoculated in the greenhouse by puncturing, but none of them showed signs of decay unless allowed to become over ripe. In the few cases where decay was noted, an examination revealed the presence of other fungi. This yeast, therefore, seems to be only weakly parasitic on tomatoes. It is able however to survive in the green fruit where asci and ascospores are formed as soon as the tomato ripens.

#### SUMMARY

Yeast-spot of lima beans and cowpeas is a hitherto undescribed disease. It was first collected in eastern Virginia in the fall of 1921.

The causal organism is apparently an undescribed species of yeast, and is described as *Nematospora phaseoli*, n. sp.

The disease appears to be restricted to the seed, on which it forms dark brown sunken areas. As a rule, the testa remains intact, but in some cases it is ruptured.

The optimum temperature for the growth of the organism in culture and for successful infection of Lima beans appears to be about 30° C.

Specimens of the disease have been collected in seven counties in eastern and central Virginia. From the severity of infection in many cases, it is likely that the disease may be of considerable economic importance.

The pathogenicity of the yeast has been demonstrated by inoculating Lima beans in the greenhouse.

VIRGINIA AGRICULTURAL EXPERIMENT STATION.

## PHYTOPATHOLOGICAL NOTES

*Plant pathology in Crimea.*—Apple scab (*Venturia inaequalis*) and pear scab (*V. pyri*) are serious diseases in Crimea as well as in the United States. The brown rot organism differentiated into *Sclerotinia cineria* and *S. fructigena* is exceedingly serious to both fruit and trees. The latter form only is supposed to be responsible for injury to trees. The writer was shown a cherry orchard at the Salgir station which was

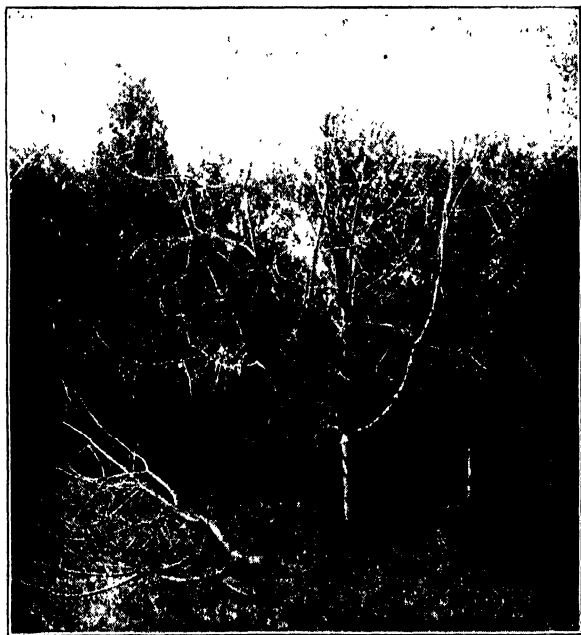


Fig. 1. Injury to fruit trees in Crimea caused by the brown rot fungus.

being killed by this organism. The fungus advances progressively from fruit to twigs and then to the main limbs and trunk much as does our pear blight organism. The accompanying photograph (Fig. 1) made under the writer's direction illustrates the method of attack and damage wrought. The disease is most serious on sweet cherries, and to trees under poor culture conditions. Such conditions as now obtain are to a large extent due to civil wars.

In the limestone country north of the Crimean mountains chlorosis is serious. It is also to be noted that this region is entirely irrigated. At the Salgir station some interesting experiments in attempting to control chlorosis by injecting chemicals have been conducted. The results are said to have been published in Russian journals. A mixture of  $\text{Fe SO}_4$ ,  $\text{K}_2\text{SO}_4$  and  $\text{Mg SO}_4$  dry and in solution were injected into trees through holes bored into the trunk. There were no untreated controls. The writer observed that above the reported point of injection the foliage was normal green, while below that point it was yellow.

The method of pruning trees has frequently been responsible for their death from heart rots and cankers. The trees in the older orchards have a large number of main branches. As a result the tops are crowded and the fruit is borne at the ends of spindling branches, which in turn reduces the yield and endangers them to breaking. The growers are now sawing off limbs five to seven inches in diameter, the wounds being left untreated. Such trees generally become infected and eventually die. *Nectria ditissima* is very serious.

Plant pests on the south slope of the mountains are frequently different from those on the north. *Gymnosporangium sabinae* is quite serious on pears. It is noteworthy that three species of Juniper are quite abundant in the mountains. *Sphaerotheca pannosa* was observed on peaches.

Insect pests cause greater damage to fruit in Crimea than fungi. The codling moth is most serious. There are many leaf-eating caterpillars and other insects not recorded in American works on fruit pests. They are particularly serious now due to the lack of insecticides.

The methods of pest control are quite backward. The sprays used when obtainable are Bordeaux mixture and Paris green. Lime-sulphur is only known as a dormant insecticide and self-boiled lime-sulphur as a spray for mildew on gooseberry. Last spring the orchardists were unable to spray because of the failure to secure shipments from Moscow of copper sulfate for the preparation of Bordeaux mixture. There are deposits of sulphur in Crimea and in the Caucasus, and abundant lime throughout Crimea so that lime-sulphur might have been made, had the growers known of its use. Power sprayers are unknown in Crimea. Indeed I was informed that there was not a power sprayer in all Russia. Small hand sprayers are used. These ordinarily are operated by a force of six men—two to pull the sprayer, two to pump, and two to spray. The pear trees north of the mountains reach a great height. The tops are sprayed from tall ladders.

The plant pathologist at Nikitski Botanical Gardens, Dr. Deckenbach, who speaks English quite well, is acquainted with American

literature. He is starting experiments in the use of lime-sulphur. The communication between scientists in Moscow and Crimea has been very poor. Dr. Deckenbach travelled north with the writer and in Moscow read his obituary notice written the year before by Prof. Jaczewski who recently visited America.

There is considerable neglect of orchards due to unavoidable disorganization incident to war and revolution but the main problem in Russia is to improve the normal pre-war methods. These methods were behind those in this country due to the backwardness of the peasants, and to the corruption, incompetence, and indifference of the former ruling class.

With the vigorous cooperation of the Russian government the scientists are making a campaign to improve the agricultural methods of Russia. They deserve all the help which American scientists can give them. They especially need scientific literature.

I found no American scientific literature in Petrograd and Moscow, for the period from 1917 to 1921 and very few publications since 1913. A few publications had found their way into Crimea during its occupation by counter-revolutionary forces. One scientist showed me two agricultural bulletins which he was guarding as though they were valuable treasures. Fortunately we now have a scientific committee at work collecting literature for the Russian scientists. I hope that American scientists will be generous in their contributions to this committee—H. W. TRUESDELL.

*Mistletoe and Smelter smoke.*—In 1921, the writer examined an area of the woodland type which for four years had been exposed to smoke from the big copper smelter at Clarkdale, Arizona. This smoke was said to contain compounds of sulphur and arsenic. Scattered over this area were many one-seeded junipers (*Juniperus monosperma*); close to the smelter the trees were dead but further away many were injured in various degrees but not yet killed. Some fifty of these living junipers had once been infected by mistletoe (*Phorodendron juniperinum*) but at the time of inspection not a single bunch of mistletoe was alive. Large and small bunches alike were dead. The mistletoe had evidently been dead for more than a year since all of the small aerial branches had fallen off leaving broken stubs some two or three inches long attached to the living juniper branches. An examination of the subcortical portions of the mistletoe showed that they were also dead.

The writer is now making a more intensive study of the mistletoe on this area with a view to determining the exact cause of its death. It is hoped that through this investigation some practical method may

be found of ridding ornamental trees of mistletoe infestation without serious injury to the trees.—W. H. LONG.

*The October number of Phytopathology was issued December 5, 1912.*

# PHYTOPATHOLOGY

VOLUME XII

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## II. SEPTORIA DISEASES OF WHEAT<sup>1</sup>

GEORGE F. WEBER

WITH PLATES XXIII TO XXXVI AND SIXTEEN FIGURES IN THE TEXT

On wheat there are two diseases caused by different species of *Septoria*, namely, *S. nodorum* Berk. and *S. tritici* Desm. The one, caused by *S. nodorum*, attacks the glumes most commonly producing brown blotches on them, hence it has been called "Glume Blotch." This usage will be followed in this paper. This disease may also attack the rachis, culms and leaves. The other disease, caused by *S. tritici*, attacks the leaves only, producing conspicuous light colored lesions in which the dark colored pycnidia are prominent and produce a speckled appearance. On account of this outstanding characteristic the name "Speckled Leaf Blotch" is here suggested for this disease. This name will be used in this paper.

### GLUME BLOTCH OF WHEAT

#### INTRODUCTION

A *Septoria* disease found upon the leaves and glumes of wheat has been reported recently as a serious disease of wheat (26). The fungus was reported present over large areas in different sections of the country, and in certain wheat growing districts serious losses in grain yield have been recorded. It is caused by the parasitic fungus *Septoria nodorum* Berk. The organism attacks different parts of the plant causing irregular shaped areas to become discolored and dead. Heads of wheat are especially seriously attacked and become blackened, beginning at the tips of the outer glumes and working toward the base.

#### THE DISEASE

##### Hosts

This organism was first found on wheat by Berkeley (2) in 1845, and by Passerini (25) in 1879. Since then it has been reported wide spread

<sup>1</sup> This is the second of three articles by the author on the *Septoria* diseases of cereals and some of the grasses. The first appeared in *Phytopathology*, number 10, volume 12.



conforming to the cultivation of the wheat plant. Sorauer (31) reported it on speltz and wheat. Neuen-Lemarie (22) listed wheat, barley, rye and oats as host plants of this organism. The writer has conducted a series of inoculation experiments in an effort to gain some knowledge as to its host range and as a result has found that only wheat species, rye and *Poa pratensis* become infected while species of barley and oats and a number of related grasses remain free from the disease. It is peculiar, however, that in the case of rye only the leaves become infected, while in the cases of wheat and *Poa pratensis* all parts above ground are susceptible. These experiments will be taken up in detail later in this paper.

### Geographical Distribution

*Septoria nodorum* Berk. was first reported in England by Berkeley (2). Later it was reported in northern Italy by Passerini (25). It was also reported in other parts of Italy by Cavara and Comes according to Voglino (36). Eriksson (12) observed this disease in Sweden. It was reported in Switzerland by Baltshauser according to Voglino (36) and from different parts of Germany by Frank (14). Selby (30) and Townsend (33) describe its appearance in Ohio and Maryland respectively. Rosen (28) reported the fungus locally in parts of Arkansas. Güssow (16) reported the disease as being general and common in Europe. Marchal and Foëx (20) found it in France near Toulouse. Sutton (32) published a short note on the occurrence of the disease in western Australia.

In the United States the disease is rather generally distributed. It has been reported as common in all wheat growing regions, which includes the Mississippi basin and smaller areas on the Atlantic and Pacific coasts. It has been reported most prevalent in the South and and East Central States of the United States.

### Economic Importance

No serious damage to the wheat crop was reported as being caused by this fungus until Townsend (33) reported serious losses in Maryland. He stated that the yield of certain fields was reduced from 30 or 35 bushels per acre to 15 bushels per acre and that the kernels were very much shrivelled. Voglino (36) observed that the wheat fields in Piedmont were often attacked by this fungus and that severely infected heads always produced shrivelled kernels of abnormal structure. Observations and notes of the disease in England by Berkeley (2) and Massee (21) state that it was abundant in the wheat fields and caused

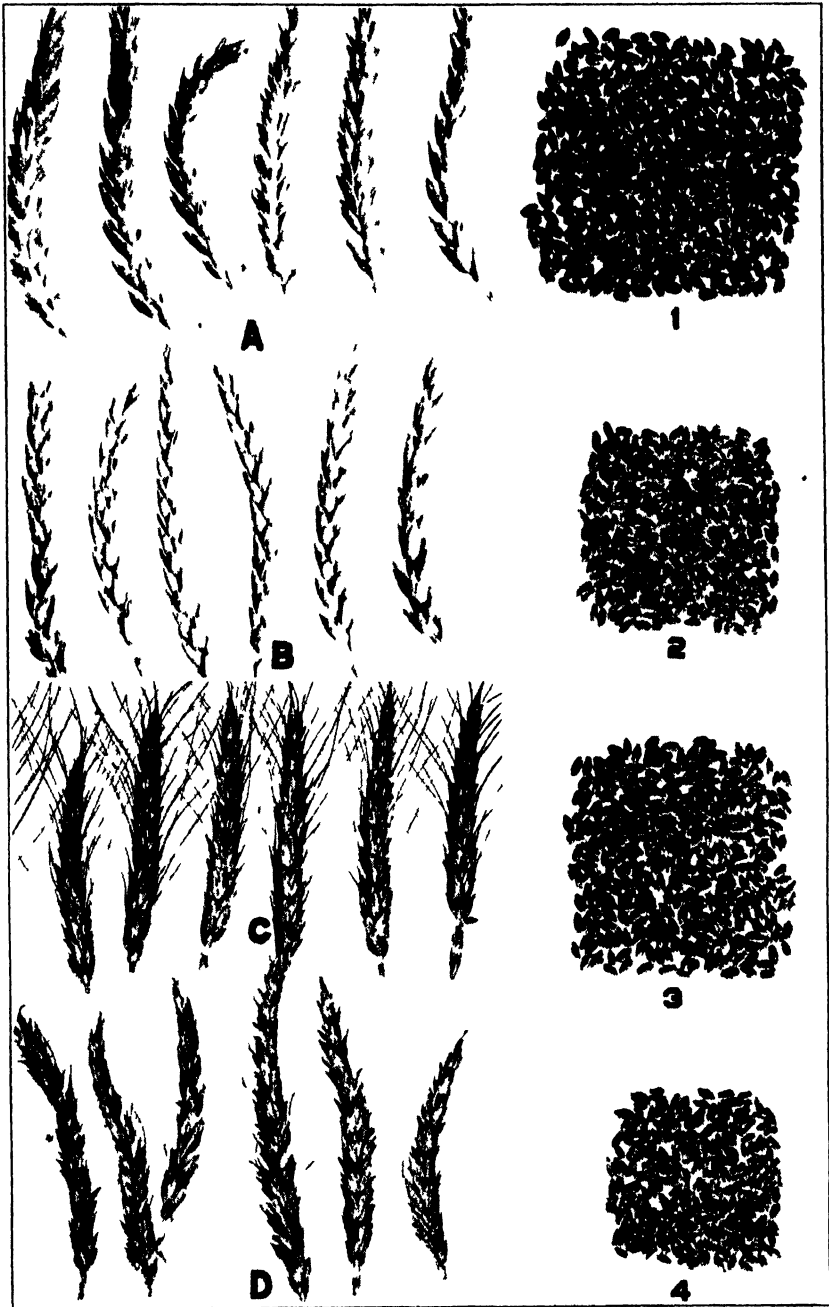


Fig. 1 Red Wave (A and B) and Turkey (C and D) wheat grown in greenhouse A and C healthy, 1 and 3 grain from similar heads of same varieties respectively B and D following inoculation with *S. nodorum*, 2 and 4 grain from heads similarly inoculated.

extensive damage. Sorauer (31) stated that the quality of the seed was badly affected by the attacks of this fungus on the host plant. Schmitz (29) reported serious damage to wheat in all parts of Italy. Güssow (16) considered no loss resulted from attacks of the fungus on wheat since it rarely penetrated to the kernels. Delacroix and Maublanc (9) report very little damage where they observed the disease. Marchal and Foëx (20) found the disease near Toulouse but reported no damage. Sutton (32) concluded that the disease was most destructive on the nodes and least destructive on the glumes, that the injury was almost wholly confined to the early sown crops, and that the disease was most serious during wet years. He did not think that the milling quality of the wheat was impaired. Rosen (28) considered this disease the most serious wheat disease in Arkansas with the exception of *Puccinia triticina* Erik. He stated that the kernels were reduced in size and shrivelled. In 1919 in Plant Disease Survey (26) a .25 per cent reduction in yield was reported from Delaware and a .5 per cent reduction from Maryland. In most states, however, the loss reported was very slight. Infection ranging from 50 to 90 per cent was reported in the Experiment Station plants in Pennsylvania. Infection was reported rather general in wheat fields east of a line from Minnesota to Arkansas. In Wisconsin the disease has been found widely scattered but has not proven serious in any locality. Figure I shows the results in yield, of controls and inoculated heads of two varieties of wheat grown in the greenhouse at Madison, Wisconsin.

### Symptoms

*Glumes and lemmas.* These parts show the first indication of infection by the appearance of small, irregular, brownish spots, usually on the upper half of the outer glume. The spots enlarge and become chocolate-brown in color and very soon black pycnidia appear scattered in the discolored areas (Fig. 2). In case of light infection only a few glumes may become diseased on a single head. In such case the others remain healthy until maturity. When the heads are badly diseased the outer and inner glumes and lemma become infected and all exposed parts become dark brown in a short time. The pycnidia are then easily observed.

*Rachis, culm and nodes.* When conditions especially favor infection these parts are attacked and become discolored. The attacked nodes of the rachis become almost black and the nodes of the culm become dark brown. Both of these parts become dotted with pycnidia. The culm is colored light brown and the spots are large, often covering the

greater part of certain internodes. Pycnidia have not been observed upon the discolored areas of the culm.

*Leaves.* Infection of the leaf resulted in the appearance of yellowish spots 8 or 9 days after inoculation. These spots become light colored and then the tissues become dry. As the drying took place pycnidia appeared very sparsely and scattered. As the central part of the spot dried out it became lighter colored. Often these spots were almost white in the center surrounded by a brownish area which marked the limit of the living tissue. Often numerous spots coalesced and when infection was heavy and growing conditions favored the development of the fungus they involved most of the leaf, killing it very soon. The leaf turned brown, the edges often curling backward. The pycnidia were rather uniformly distributed over both surfaces of the leaf and upon close examination were found to be seriatly arranged. The pycnidia on the leaves were in every way similar to those found on the glumes, rachis, and nodes.

#### CAUSAL ORGANISM

##### Taxonomy

This disease was first described by Berkeley (2) on the nodes and internodes of wheat and the causal organism named *Septoria nodorum*. Passerini (24) described a disease on the glumes of wheat in Parma, Italy, and named the organism *Septoria glumarum*. The latter binomial has been generally accepted and is in common use in literature at present. Kühn (18) described an organism on the glumes of wheat in Germany which he named *Phoma hennebergii*. Upon examination of this material later Berlese and Voglino (3) found the organism to be *Macrophoma* (*Cylindrophoma*) *hennebergii*. Voglino (36) stated that Frank and Kirchner reported the disease as common in Germany, and also that Kirchner and Boltshauser considered it very similar to *Septoria glumarum* Pass. Because of the resemblance between Kühn's description and the description of Passerini, Voglino, although unable to examine the specimen of Kühn, thought that *Macrophoma* (*Cylindrophoma*) *hennebergii*. Kühn should be included with *Septoria glumarum* Pass. Grove (15) examined this disease on wheat from Australia and England and later compared these specimens with Berkeley's original collection in Kew herbarium. He concluded that the diseases on culm, node and glumes were caused by the same species of fungus and that the diseased material from England and Australia was the same as that which Berkeley deposited in Kew. Rosen (28) stated that the disease found on wheat in

Arkansas conformed to Groves' description. The fungus found widely distributed in the United States is undoubtedly *Septoria nodorum* Berk. Hence the nomenclature and synonyms are as follows:

*Septoria nodorum* Berk.

*Septoria glumarum* Pass.

*Phoma hennebergii* Kühn

*Macrophoma* (*Cylindrophoma*) *hennebergii* Berk. and Vog.



Fig. 2. Heads of "Red Wave" wheat heavily infected with *S. nodorum* Berk. Note numerous pycnidia on glumes.

*Mycelium.* The mycelium is colorless, 2-3 $\mu$  thick, septate and very much branched. The walls are thin, and large vacuoles often appear in the older strands. In colonies on potato agar it is white, then light-olivaceous areas appear, and after a couple of weeks the surface of the media is almost black. In the host plants the mycelium grows rapidly and forms in clumps when forming the pycnidia.

*Conidia.* (Fig. 3, A.) Found only on oatmeal agar; oblong, cylindrical, entire, straight or slightly curved, rounded ends, hyaline, 3-septate, guttulate near septa, 2-4 x 18-32 $\mu$ , averaging 3 x 24 $\mu$ ; produced laterally usually at the septa of germinating pycnosporos.

*Pycnidia.* Pycnidia are seriatly arranged or widely scattered, spherical, flattened or elongate, subepidermal, ranging in size from 160 to 210 $\mu$  in diameter. The wall is thin, soft, and parenchymatous, at first light brown in color, later becoming dark brown or black. The ostiole is circular to oval opening between the guard cells of the stomata (Fig. 4).

*Pycnosporos.* (Fig. 3 B, C.) Pycnosporos are oblong, cylindrical, straight, curved or angularly bent, obtuse and rounded at the ends, hyaline, 3-septate, guttulate, 2-4 x 18-32 $\mu$ , averaging 3 x 26 $\mu$ . They emerge from the pycnidium in long serpentine strands. The spores are repelled from each other probably by surface tension phenomena.

*Perithecia.* This stage of the life history of the organism is imperfectly known. Voglino (36) cultured *Septoria nodorum* Berk. and succeeded in discovering perithecia in certain cultures that contained asci and ascospores which were hyaline and one septate. On the basis of this he identified the fungus as *Sphaerella exitialis* Morini. However, there seems to be some question if the ascospores may not have been somewhat immature. At least the evidence as brought out later indicates that the ascigerous stage may be a species of *Leptosphaeria*.

Davis (8) found some perithecia associated with pycnidia of *S. nodorum* which he described as having three-septate, hyaline spores and paraphyses, thus placing the organism in the genus *Sphaerulina*. The writer found some perithecia closely associated with pycnidia of *S. nodorum* Berk. on glumes of Red Wave Wheat in July, 1921 (Pl. XXXIII), and upon examination found the ascospores were three-septate and of a yellow olivaceous color which placed the fungus in the genus *Leptosphaeria*.

Through the kindness of Dr. J. J. Davis some of the material collected by him in 1916 was examined, also some of his permanent mounts of the fruiting bodies. In these mounts, structures similar to paraphyses were observed and examination of perithecia from the wheat leaves

collected by him also showed that paraphyses were present there (Fig. 5A and B). Accordingly, the organism should be placed in the family Pleosporaceae rather than in the family Mycosphaerellaceae, and in either the genus *Metasphaeria* or *Leptosphaeria* rather than in the genus *Sphaerulina*. The ascospores appeared hyaline and would consequently be placed in the genus *Metasphaeria*. However, comparisons were made with other species of *Metasphaeria* and *Leptosphaeria* in the herbarium and these ascospores were doubtfully hyaline. When it is considered that ascospores comparable to these in every way, except that they were colored, were found by the writer associated with pycnidia of *Septoria nodorum* Berk. it is quite probable that the fruiting bodies collected in 1916 by Dr. Davis were somewhat immature, hence not yet colored. Therefore, this form is probably a species of *Leptosphaeria*. The relation between this species and *Septoria nodorum* Berk. was not definitely proved because the ascospores were not viable, and on this account neither isolations nor inoculation experiments could be made.

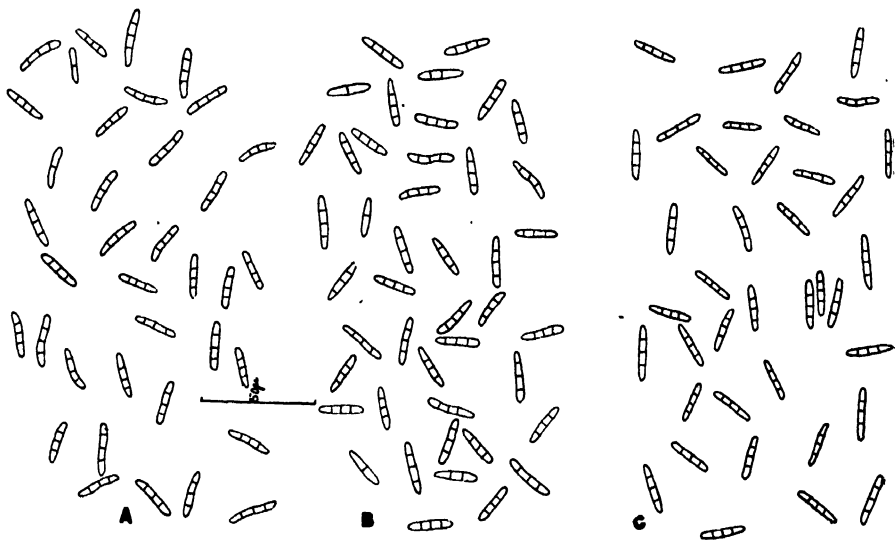


Fig. 3. Camera lucida drawings of spores of *Septoria nodorum* Berk. A. Conidia grown in culture. B. Pycnosporos grown in culture. C. Pycnosporos collected in the field.

The perithecia found by the writer in July, 1921, in the glumes of Red Wave wheat, were subepidermal, globose to subglobose, 40 to 140  $\mu$  in diameter. The walls were thin, parenchymatous, black, and slightly raised. Ostiole circular to oval 18 to 24  $\mu$  in diameter. Asci

cylindrical, hyaline, thin walled, rounded at end, eight-spored. Ascospores fusoid, ends rounded, 3-septate, slightly constricted at central septum, yellow to brown in color, 5 to 6 by 20 to 30 $\mu$ . Paraphyses, cylindrical, rounded at tips, hyaline, septate, slightly longer than asci.

### Physiology

*Cultural studies.* The organism was obtained in pure culture by isolating pycnospores. The pycnospores were obtained from the pycnidia in the glumes by soaking the infected glumes in water for a short period during which the pycnospores exuded through the ostioles in long threads. Single pycnospores were isolated on potato agar plates

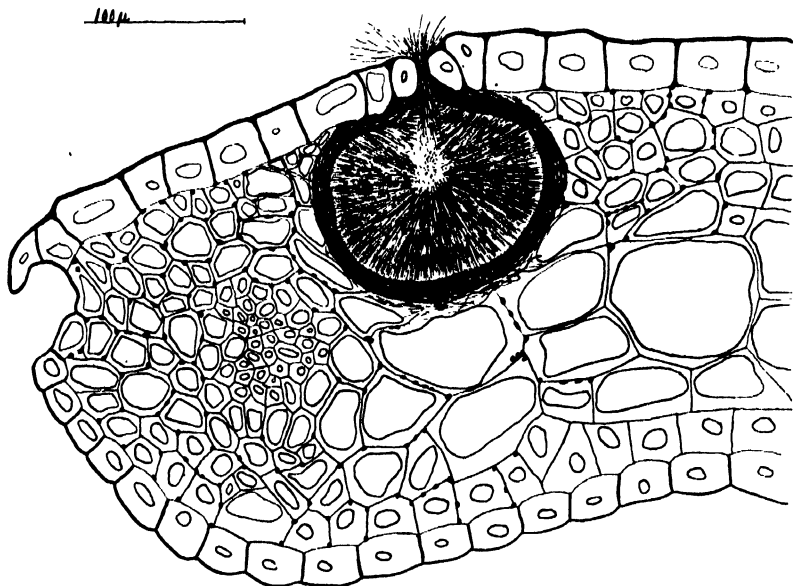


Fig. 4. Camera lucida drawing of a cross section of a pycnidium of *Septoria nodorum* Berk. Note ostiole under stomata. The black dots between the host cells are cross sections of fungus mycelium.

and upon germination were transferred to culture tubes for growth. The colonies developed into white masses of mycelium which grew quite rapidly making a thick, vigorous looking mat of mycelium. A number of different media were used as a source of nutrition for the fungus. Luxuriant growth was obtained on potato-dextrose agar, oatmeal-agar, wheat-extract agar, sterile oatmeal, cornmeal, bran, barley, wheat, sweet clover stems, alfalfa stems, wheat stems and wheat heads. Poorer growth was observed on Lima-bean agar and string-bean agar. The



potato-dextrose agar and oatmeal agar proved to be the best media, however, for the development of the mycelium and for the production of pycnidia. The pycnidia, which were produced only on these two media, appeared slightly imbedded in the media under the mycelium.

Pycnidia of *Septoria nodorum* were collected by the writer in July, 1921, at Madison, Wisconsin, on Red Wave wheat heads. In November, 1921, the pycnosporos were obtained and germinated. Single germin-

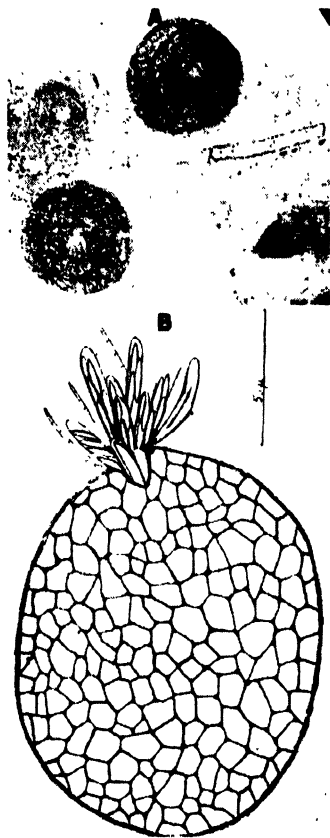


Fig. 5. A. Photomicrograph of perithecia found on a wheat leaf intermixed with pycnidia of *S. nodorum* Berk. B. Camera lucida drawing of a perithecium shown in A.

ating spores were transferred to potato-dextrose agar slants for growth. In February, 1922, pycnidia containing pycnosporos were discovered in many of the cultures. These pycnosporos were germinated on potato-dextrose agar and oatmeal agar slants. A week later examination of the cultures showed that on the surface of 10 out of 14 of the oatmeal

agar cultres conidia wete forming. They were scattered over the surface of the slant in small salmon-pink, circular, raised colonies (Pl. XXXIII, C). They compared favorably with pycnospores collected in

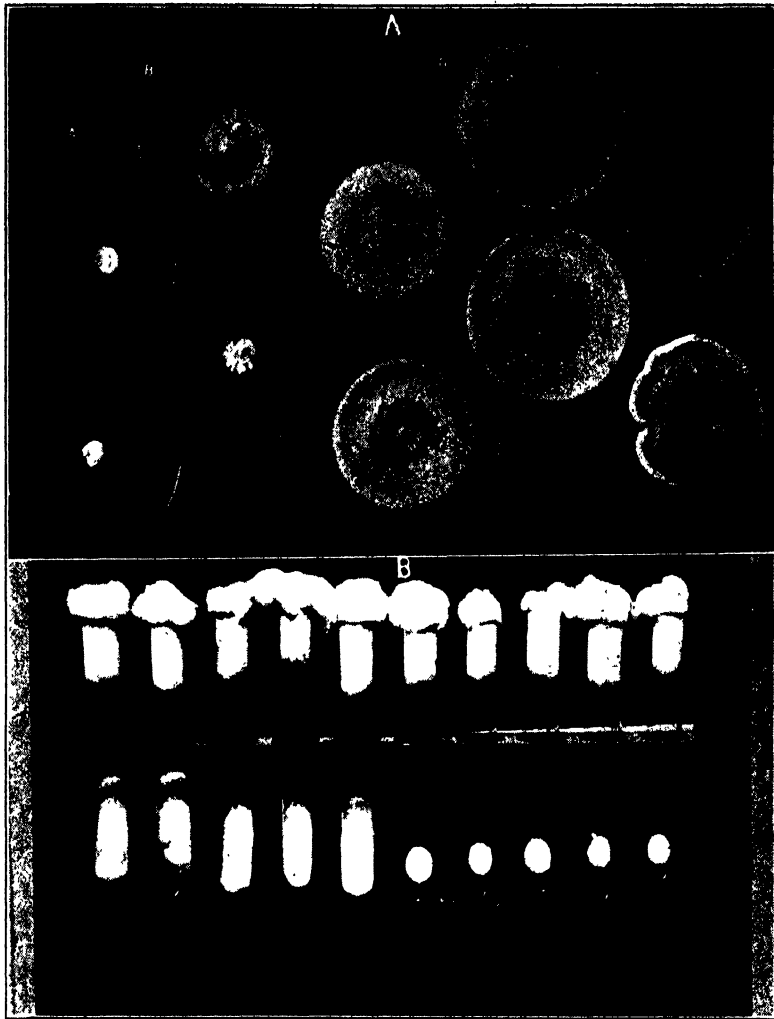


Fig. 6. A. Photomicrograph of *S. nodorum* after growing in plate cultures for 14 days at the following temperatures: A. 4-6° C.; B. 8-10° C.; C. 14-16° C.; D. 20-24° C.; E. 28-32° C.

B. Growth of mycelium of *S. nodorum* Berk. on potato dextrose agar adjusted to pH 6.2 and pH 7.8 grown 10 days at room temperature.

the field as to size, septation, and shape. None of the transfers to other media developed conidia.

Conidia which were grown on oatmeal agar were transferred to a number of tubes of potato-dextrose agar and in every instance germinated by sending out germ tubes which branched profusely and formed a mycelial colony. After about one week numerous pycnidia began to form under the mycelium; the pycnospores were normal as to size, shape and septation.

*Spore germination.* The pycnospores of *Septoria nodorum* Berk. germinated best in water. In water and on agar poured-plates they formed germ tubes from one or more cells of the spore. The germ tubes branched and the colony developed into a white mass of mycelium. Voglino(36) stated that he occasionally secured conidia from the pycnospores when they were germinated in water. He shows figures of conidia formed from pycnospores. They were formed by the budding process very similar to that observed by the writer in a number of species of *Septoria*.

*Ascospores.* The ascospores of this organism have not been germinated by the writer. Voglino (36) described the germination of the ascospores of *Sphaerella exitialis* Morini, which he concluded were the perfect form of *Septoria nodorum* Berk. When he inoculated wheat plants with them pycnidia were developed. The ascospores germinate readily in water, they developed a short germination tube and then formed conidia on the sides of the germination tube.

*Temperature relations.* The pycnospores germinated best at about 20° C.; lower temperatures tended to retard the process, while at higher temperatures spore germination was either irregular in formation or prohibited altogether.

The mycelium growing on potato agar made the best growth between 20–24° C. The growth diminished toward the lower temperatures, very little growth taking place at 4° C. At 30° C. the growth was concentrically zoned and reddish-purple in color. Above 30° C. the growth was scant (Fig. 7 A).

*Relation of reaction of media to mycelial growth.* Bits of mycelium were transferred to potato agar slants which had been made to test pH 6.2 and pH 7.8. The fungus was grown for 12 days at room temperature. The difference in growth is shown in figure 6 B. Mycelial growth was greatly favored by a slightly acid medium.

#### Pathogenicity

Voglino (36) made a limited number of inoculation experiments on the different parts of the wheat plant with pycnospores of *S. nodorum* (*S. glumarum*) and found that the organism was pathogenic upon all parts of the wheat plant above ground. i. e., nodes, internodes, rachis,

glumes, awns, and leaves. Rosen (28) made some inoculations in the greenhouse and reported leaf and glume infections. In the investigations conducted by the writer all parts of the wheat plant have been infected by inoculation by pycnosporos both in the greenhouse and in the field.

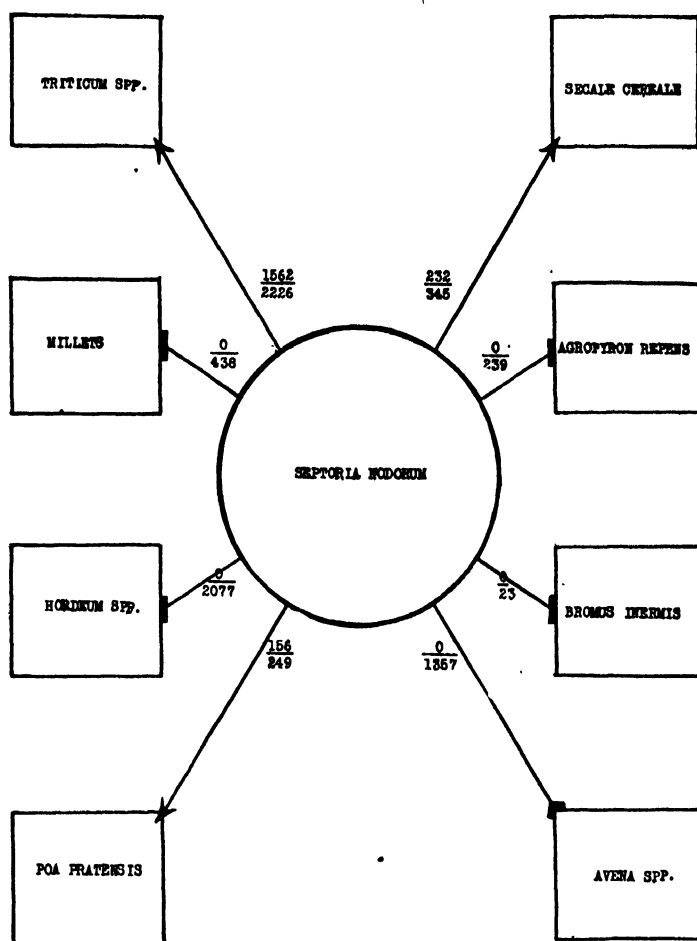


Fig. 7. Diagram showing source of inoculum (in the circle) and the hosts inoculated (in the rectangles). The denominator of the fraction designates the number of leaves inoculated and the numerator the number of leaves infected at the end of 14 days. Arrows alone indicate infections.

The pycnosporos isolated from any part of the plant were identical in every way. Inoculation experiments were conducted by employing such isolated pycnosporos. The disease was reproduced after two weeks showing up characteristically on the different parts of the plants. The

leaves, when inoculations were made in the field, became infected more readily than other parts of the plant. The reverse was found to be true with plants inoculated in the greenhouse. The glumes of plants inoculated in the greenhouse showed signs of the disease first, followed by a browning of the rachis and the upper internode. The organism was reisolated from the artificially produced diseased areas and it was similar in every respect to the one with which the inoculations were made.

*Cross inoculations.* In the review of the literature the host range of this organism was found to include practically all of the cereals. Experiments were conducted by the writer, in which 47 species of varieties of cereals and grasses were inoculated with pycnosporos suspended in water.

The inoculum was obtained from wheat glumes and leaves collected in the field and from wheat glumes upon which the disease was artificially produced in the greenhouse. The pycnosporos which exuded from the pycnidia of infected glumes soaked in water formed a spore suspension which was sprayed upon the plants with an atomizer. The inoculated parts were then covered with paper bags which were removed after from 48 to 72 hours. A summary of the data from these experiments is given in table 1 and is still further summarized diagrammatically in figure 7.

TABLE 1

*Summary of results from inoculating various grains and grasses with pycnosporos of Septoria nodorum Berk.*

Hosts	Source of inoculum <sup>1</sup>	Number of leaves	
		Inoculated	Diseased
<i>Avena barbata</i>	1	77	0
	2	17	0
	3	15	0
<i>Avena brevis</i>	1	98	0
	2	19	0
	3	19	0
<i>Avena fatua</i>	1	110	0
	2	42	0
	3	19	0
<i>Avena nuda chinensis</i>	1	79	0
	2	12	0
	3	17	0

<sup>1</sup> Sources of inoculum as designated in table 3 are as follows: 1. Wheat heads and leaves. 2. Rye leaves. 3. *Poa pratensis* panicles and leaves.

Hosts	Source of inoculum <sup>1</sup>	Number of leaves	
		Inoculated	Diseased
<i>Avena sativa</i> (Culbertson)	1	100	0
	2	54	0
	3	16	0
<i>Avena sativa nigra</i>	1	105	0
	2	14	0
	3	14	0
<i>Avena sativa orientalis</i>	1	79	0
	2	20	0
	3	20	0
<i>Avena sterilis</i> (a)	1	100	0
	2	11	0
	3	19	0
<i>Avena sterilis</i> (b)	1	100	0
	2	19	0
	3	18	0
<i>Avena strigosa</i>	1	103	0
	2	15	0
	3	16	0
<i>Hordeum deficiens</i>	1	94	0
	2	16	0
	3	22	0
<i>Hordeum horsfordianum</i> (Ore.)	1	102	0
	2	19	0
	3	22	0
<i>Hordeum horsfordianum</i> (S. Dak.)	1	99	0
	2	13	0
	3	22	0
<i>Hordeum distichon erectum</i>	1	100	0
	2	19	0
	3	21	0
<i>Hordeum distichon</i>	1	97	0
	2	8	0
	3	25	0

Hosts	Source of inoculum <sup>1</sup>	Number of leaves	
		Inoculated	Diseased
<i>Hordeum distichon nutans</i>	1	100	0
	2	58	0
	3	14	0
<i>Hordeum vulgare coerulescens</i>	1	69	0
	2	20	0
	3	22	0
<i>Hordeum vulgare hexastrichum</i>	1	104	0
	2	17	0
	3	15	0
<i>Hordeum vulgare himalaya</i>	1	151	0
	2	15	0
	3	26	0
<i>Hordeum vulgare nigrum</i>	1	106	0
	2	10	0
	3	19	0
<i>Hordeum vulgare pallidum</i>	1	113	0
	2	19	0
	3	18	0
<i>Hordeum vulgare trifurcatum</i>	1	193	0
	2	34	0
	3	38	0
<i>Triticum aestivum</i> (Marquis)	1	135	121
	2	62	58
	3	17	12
<i>Triticum aest. lutescens</i> (Harvest Queen)	1	100	23
	2	76	54
	3	18	14
<i>Triticum aest. lutescens</i> (Early May)	1	109	81
	2	55	20
	3	15	8
<i>Triticum aest. miltura</i> (Red Wave)	1	107	81
	2	59	39
	3	20	15

Hosts	Source of inoculum <sup>1</sup>	Number of leaves	
		Inoculated	Diseased
<i>Triticum compactum</i>	1	91	86
	2	48	28
	3	21	11
<i>Triticum dicoccum barrum</i>	1	88	71
	2	47	12
	3	24	12
<i>Triticum dicoccum atratum</i>	1	118	96
	2	21	14
	3	19	13
<i>Triticum durum</i>	1	90	74
	2	46	29
	3	21	11
<i>Triticum monococcum</i>	1	92	74
	2	52	21
	3	16	14
<i>Triticum polonicum</i>	1	95	82
	2	62	29
	3	15	8
<i>Triticum spelta</i>	1	132	119
	2	63	29
	3	21	18
<i>Triticum turgidum</i>	1	104	84
	2	33	23
	3	22	8
<i>Secale cereale</i>	1	225	150
	2	83	62
	3	37	20
<i>Agropyron repens</i>	1	137	0
	2	73	0
	3	24	0
<i>Bromus inermis</i>	1	23	0
	2	0	0
	3	0	0



Hosts	Source of inoculum <sup>1</sup>	Number of leaves	
		Inoculated	Diseased
<i>Chaetochloa italica</i>	1	37	0
	2	8	0
	3	16	0
<i>Chaetochloa viridis</i>	1	85	0
	2	16	0
	3	8	0
<i>Echinochloa crus-galli</i>	1	85	0
	2	16	0
	3	8	0
<i>Panicum clandestinum</i>	1	69	0
	2	10	0
	3	17	0
<i>Pennisetum glaucum</i>	1	56	0
	2	12	0
	3	18	0
<i>Poa pratensis</i>	1	192	72
	2	24	10
	3	21	14

It will be noted from the table that *Septoria modorum* will attack the wheat species, rye and blue grass, regardless of whether or not the inoculum was obtained from any one of the three hosts mentioned.

#### Seasonal Development

*On leaves.*—Infection takes place on the leaves at any time during the spring, summer or fall. In the early spring and late fall when the temperature usually falls to freezing during the night and the days are comparatively warm, characteristic lesions on the leaves may be found; they are more or less circular, lighter colored and sparsely dotted with pycnidia. The pycnidia developed in the fall, contained pycnosporos that germinated almost 100 per cent in the spring. The lesions formed in the spring slowly invaded the whole leaf and killed it. When the tillers began to shoot infections were observed on all leaves over two weeks old.

*Heads.*—Infection was not observed on the head until shortly after blossoming time which gave approximately a sixteen day incubation

period for the fungus providing inoculation took place from one to three days after the heads appeared out of the sheath. Infection on the heads was not confined to any definite month but rather took place at a certain stage of the development of the wheat plant. In the vicinity of Madison, Wisconsin, June and July were the two months during which most of the wheat heads become infected. The disease spreads rapidly from the time of first infection until the grain is cut.

### Source of Inoculum

The sources of the inoculum are the pycnidia which are formed in the diseased areas on leaves, nodes, glumes and awns. The pycnosporos exude readily when the pycnidia are held under moist conditions. Germination of the pycnosporos takes place in a very few hours after they escape from the pycnidia.

### Modes of Overwintering and Longevity of Spores

The pycnosporos of the fungus live over the winter period, provided they are retained in the pycnidium. During the winter of 1920-21 some wheat heads, heavily infected with this disease, were placed out-of-doors in the early fall and spore germination tests were made twice a month from November, 1920, until May 1, 1921. The results showed that the spores retained germinating powers during the whole period with little or no variation. A series of tests were made from similar material collected at the same time and kept in the laboratory. The germination of the spores from the laboratory material decreased 40 to 50 per cent after four months and at the end of six months very little germination was obtained. More than 30 per cent of the spores from material placed out-of-doors showed germination after 18 months.

### Modes of Dissemination

The fungus is no doubt distributed most extensively by the dissemination of the pycnidia in diseased tissue. Locally the spread is by means of pycnosporos. The pycnidia are carried about on the straw and chaff by means of the wind and other agencies at threshing time and on the straw transported for feed and commercial uses. A large amount of diseased plant material is usually left in the field after cutting. This material disintegrates during the winter, liberating the pycnidia which are disseminated by such agencies as wind, rain, and flowing water. The pycnosporos are killed very easily by drying and by direct sunlight so that when dry they play a very small part in the dissemination of the fungus.

### Time of Natural Infection

The leaves become infected at almost any time after they unfold. Infection is most prevalent, however, during April, May, and October on the leaves, and June and July on the glumes.

### Mode of Infection

The fungus enters the host plant by direct penetration of the cuticle.

It then grows in between the epidermal cells into the parenchymatous tissue.

The pycnospores which become lodged on the epidermis usually lie lengthwise in the depressions directly above adjacent epidermal cells. They germinate and the germ tubes follow the depression for a short distance, then stop abruptly and penetrate the cuticle.

### Period of Incubation

Under both field and greenhouse conditions it was found that the time from inoculation until the disease was produced, (bearing mature fruiting bodies) was from 12 to 16 days. Six or seven days after inoculation very faint blotches of slightly lighter colored tissue were observed scattered over the heretofore healthy leaf. These blotches became lighter and lighter after about the tenth day when they were quite yellow. At this time light brown dots, the young pycnidia appeared which enlarged and became black, in which condition they were mature. Occasionally more than 14 days were required for the production of mature pycnidia, but in these cases the temperature was below the optimum for the development of the fungus. The incubation period on the leaves and glumes was about the same while on the culms, nodes, and rachis it was a few days longer.

### Pathological Anatomy

The hyphae which grow through the cuticle are very small. They enlarge somewhat after the cuticle is penetrated. They are about natural size when they grow between the epidermal cells. Branching of the hyphae was not observed until after they had passed through the epidermis and come in contact with the parenchymatous cells beneath. The hyphae usually branched and changed direction of growth at this point, extending in all directions on a plane parallel to the epidermal layer. Long strands were observed in the crevices formed by the epidermal cells running parallel with the vascular bundles in the leaf. Small branches developed perpendicularly and extended between the parenchymatous cells. Often a single host cell was formed, completely

encircled by several hyphae. Aggregations of hyphae in various stages of density were found in the substomatal spaces so that it was possible to follow the formation of the pycnidia in their development from a few strands of hyphae to the mature fruiting body. The pycnidia were found to develop only in the substomatal chamber with their ostioles directly under the stomata. The host cells adjacent to the pycnidia were usually obscured by the dense wefts of hyphae. Several layers of cells in close association with the pycnidia were dead and obscured in outline. The host cells in the vicinity of the invading hyphae appeared to be in a normal condition. They showed no effects of the parasite until almost all of the intercellular spaces contained numerous hyphae. The hyphae invaded the culm but no fruiting bodies were found in the internodes. They were found commonly on the nodes, however.

#### Effects on the Physiology of the Host.

Wheat plants which were severely infected with this organism were stunted in growth. Most of the tillers were killed when small, leaving only two or three to head. The heads were from  $\frac{1}{3}$  to  $\frac{1}{2}$  normal size and usually empty or contained only a few shrunken kernels. Light infection on the glumes did not materially affect the host as far as yield was concerned. Severe infection, however, when all the glumes were involved reduced the yield 50 per cent or more. The kernels that were formed were shrunken and light in weight. Leaf infection in severe cases reduced the healthy leaf area and as a consequence reduced the production of carbohydrates. Only when practically all the leaves were involved was the yield noticeably reduced. Infection on the nodes was thought by Sutton (32) to result in the most severe losses. He stated that the mycelium entered the vessels and plunged them up thus cutting off the upward flow of sap. As a result of node infection two conditions depending upon the time of infection arise. Severe leaf infection generally spreads to the nodes which results in the stunting of the plant and a loss in yield. When glume infection precedes node infection the plants are fully grown so that there is no loss of yield unless the glume infection is severe.

#### HOST VARIETAL RELATIONS

Vogolino (52) conducted an inoculation experiment in which he sprayed pycnosporos of *Septoria nodorum* (*S. glumarum*) on the heads of three kinds of wheat, namely, "Nostrale" a susceptible variety, and "Noe" and "Petaniello" resistant varieties. The "Nostrale," became heavily infected while the "Noe" and "Petaniello" remained free from the

disease. He explained that this resistance in the one case was due to the change of chlorophyll bearing tissue to mechanical tissue, and the germ tubes not finding a suitable substratum died.

In the investigations conducted by the writer all the species of *Triticum* were found susceptible to the disease, and species of *T. durum*, *T. aestivum*, *T. compactum*, *T. turgidum*, and *T. polonicum* were found more susceptible, however, than *T. spelta*, *T. monococcum* and *T. dicoccum*. The several varieties of *T. aestivum* showed no marked difference in susceptibility, no outstanding cases of resistance being noted. The different varieties of the other species were not grown.

#### SUMMARY

1. *Septoria nodorum* Berk. is widely distributed in wheat growing regions and is pathogenic on the heads, leaves and culms of wheat species and *Poa pratensis*, and leaves of *Secale cereale*.

2. The disease is of marked economic importance more or less locally. The symptoms are distinctive on the glumes and culms and somewhat obscure on the leaves unless pycnidia are present.

3. The fungus grows readily on various artificial culture media, but especially well on potato-dextrose agar. Mycelium, conidia, and pycnidia have been developed in culture.

4. The cardinal temperatures for growth are as follows: minimum 4° C.; optimum 20 to 24° C.; maximum 32° C.

5. The pycnospores if enclosed in the pycnidium remain viable over winter in the vicinity of Madison, Wisconsin.

6. The fungus enters the host by direct penetration of the cuticle.

7. Period of incubation is from 12 to 15 days. The hyphae are intercellular and the pycnidia are subepidermal under the stomata.

8. Severely infected plants are usually stunted, the culms are weak, and the heads are about one-half normal size.

9. All species of *Triticum* inoculated were found to be susceptible. *T. spelta*, *T. monococcum*, and *T. dicoccum* were not as susceptible, however, as the other five species that were inoculated.

## SPECKLED LEAF BLOTCH OF WHEAT

#### INTRODUCTION

A disease of wheat widely distributed and commonly known as "Leaf spot" or "Nebular leaf spot" caused by *Septoria tritici* Desm., is here called "Speckled leaf blotch" of wheat. This name is chosen on account of the characteristic, speckled appearance of the lesions caused by the

presence of the dark brown or black pycnidia in the light colored, dead leaf tissues of the lesions. This disease which is reported widespread in Europe, Asia, Australia, and America was found to be prevalent in the vicinity of Madison, Wisconsin. Observations made during the fall of 1920 in 50 different fields showed that every field of winter wheat visited was infected with this disease. The per cent of infected plants ranged from 10 to 100 per cent. It was found that a large number of seedlings were killed in fields where infection was severe. During the winter and spring many tillers were also killed; in these instances all of the leaf surface of the tiller was covered with pycnidia. The severity of this disease and the uncertainty of its relationship to similar *Septoria* diseases on cereals prompted these investigations.

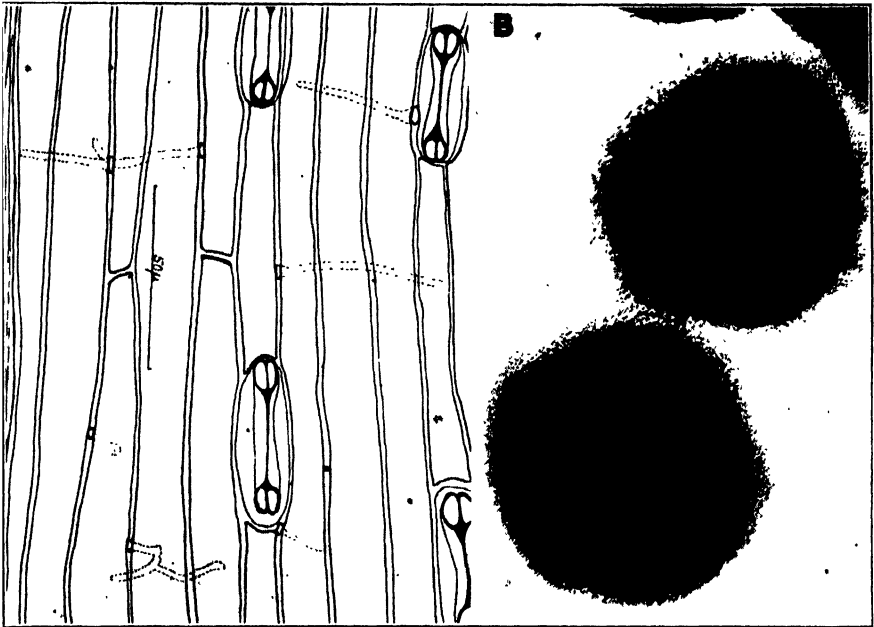


Fig. 8. A. Camera lucida drawing of surface of inoculated wheat leaf showing points of penetration by *S. tritici* and extent of growth at the end of about 60 hours.  $\times 700$ .

B. Photomicrograph of two seven days old colonies of *S. tritici* on potato-dextrose agar.

#### THE DISEASE

##### Hosts

The host range of *Septoria tritici* Desm. is more or less limited, especially among the cereals. Desmazieres (10) first reported it on wheat

and Frank (14) reported it on "new grain" in November, as doing serious damage. Cavara (5) found the diseases on wheat doing extensive damage. Prillieux (27) and Tubeuf (34) both report the disease on wheat, Tubeuf having also found it on rye and certain wild grasses which he did not list by name. Pammel (23) and Cooke (6) report the disease in the United States on wheat and certain wild grasses. Sorauer



Fig. 9. A. Three month old winter wheat plant showing leaves (grouped to the right) killed by the fungus. B. Winter wheat plant showing the infected leaves tied in bundles November 1, 1920, also the small portions of the leaves that remained on April 1, 1921.

(31), Neuen-Lemarie (22), Ferrais (13) and Delacroix and Maublanc (9) report the disease at different times in different parts of Europe on wheat species only. Butler (4) reported the disease on wheat and some

wild grasses. Beach (1) reported the disease only on wheat species and certain grasses and stated that the disease would not go to rye, oats, or barley. The writer has conducted numerous experiments both in the field and in the greenhouse in an effort to determine further the host range. Up to the present time the hosts found susceptible to the disease are the wheat species, rye, and *Poa pratensis*. More than four thousand leaves of oats and barley of different species and varieties have been inoculated under various conditions of moisture and temperature, and at different times of the year, and in no case has the disease ever been reproduced on any of these species or varieties.

### Geographical Distribution

This disease has been reported from most of the wheat growing regions of the world. Desmazieres (10) first described it from diseased wheat in France. Prillieux (27), Neuven-Lemarie (22), and Ferrais (13) all report the disease from parts of France. Passerini (24), Frank (14), Cavara (5), and Ferrais (13) report this disease from Italy. Frank (14) and Sorauer (31) report it from parts of Germany. Cooke (6), and Ferrais (13) report it from England, and Butler (4) reports it from parts of India and Australia.

The disease is well distributed over the United States. It was first reported by Pammel (23) and since that time by almost every experiment station in wheat growing districts. Beach (1) reports it very extensively around Urbana, Illinois. The writer has found this disease in every wheat field inspected in the vicinity of Madison, Wisconsin, and the causal fungus has been found to be in a viable condition on wheat plants at all times of the year.

### Economic Importance

It is a difficult task to determine the loss caused by this disease. Only scattering reports can be found in the literature such as those given by Frank (14), Cavara (5), and Prillieux (27) who report serious losses from this disease in northern Italy. Butler (4) states that the disease does slight damages in India. In the United States no attempt has been made to distinguish between the diseases caused by *Septoria tritici* Desm. and *Septoria graminum* Desm. As a result, all reports of losses are published under the heading of *Septoria* leaf spot. The loss has been found to be greatest on seedlings in the fall and on tillers in early spring, 5 per cent of which have been found killed by this disease. The losses from the disease on other parts of the plant later in the season is negligible.



## Symptoms

The symptoms are somewhat variable. In the fall when the disease first appears on the wheat seedlings it is conspicuous as more or less circular or oval irregular (nebula-like) spots scattered over the blades of the leaves. The center is usually somewhat light colored but still greenish and it shades off into the natural color of the leaf. The spot

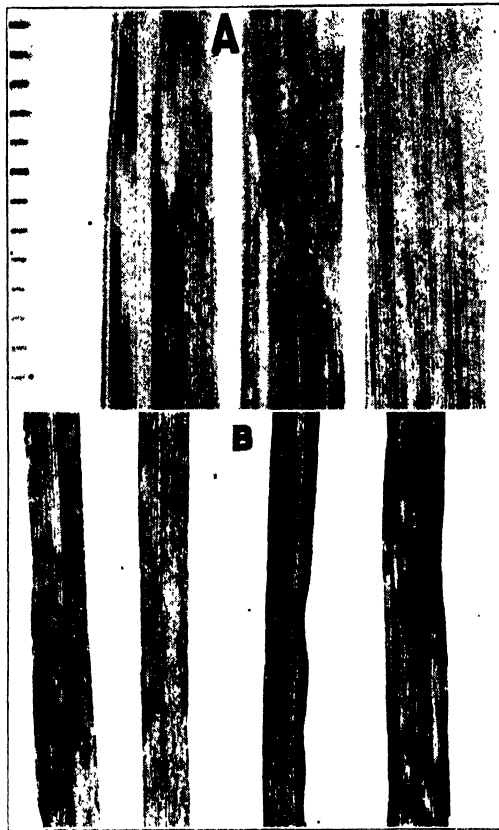


Fig. 10. B. Lesions (showing mottling and blotching as well as pycnidia) resulting from artificial inoculation in the greenhouse with pycnosporos. A. Portions of heavily infected wheat leaves showing the mature pycnidia of *S. tritici*.

does not stand out prominently and were it not thickly dotted with prominent black pycnidia it would be almost unnoticeable (Pl. XXXIV). The disease remains in this form on the green leaves. It gradually spreads and finally involves the whole leaf blade by coalescing with other infection spots. Heavily infected leaves studded with pycnidia (Fig. 9 A) died prematurely. As the new leaves develop they become infected

also. In the spring after growth starts the spots gradually change to a more elongate or linear form, becoming reddish-brown in color. They finally become somewhat light colored in the older areas (Fig. 10). The spot is at first more or less delimited by the larger veins. The entire leaf is finally involved. In certain severe cases of infection, there was not a square centimeter of healthy leaf blade tissue at blossoming time. The flag leaf and the next three leaves down the culm become completely involved in a very short time after once infected. They were prematurely killed, of a characteristic brown color, hanging down from the ligule; they became hard and dry and curled backward from each margin.

In the greenhouse the first symptoms that were detected on an inoculated plant appeared on the eighth day. At this time a slight, barely noticeable mottling of the previously uniform, green color took place. On the ninth day a decided yellowing was evident. After ten or eleven days the central portion of the light colored spot began to die and turn brown. At this time small, very light brown dots were detected in the lighter areas of the leaf, with the aid of a hand lens. After thirteen days the central portion of the spot was completely killed (Fig. 10 A), the pycnidia being plainly visible as brown dots. This is a description of one extreme, the other extreme is that the color of the infected areas remained green while the areas between the places of infection became yellow, light colored and finally died leaving the infected areas as "green islands" on the apparently dead leaf blade. These two extremes and all variations between were involved in the reproduction of the disease in the greenhouse.

#### CAUSAL ORGANISM

##### Taxonomy

*Septoria tritici* Desm. was described on wheat in 1842 by Desmazieres (10) in France. He gave a complete description of the organism and the disease. The next year he (11) described *S. graminum* on dry leaves of grasses (*in foliis siccis graminum*). This he described as very similar to *S. tritici*. In fact he regards these two species so similar as to include them later (12) under the same specific name, reducing *S. tritici* to varietal rank under the specific name *graminum*. This usage was subsequently followed by others and still others (5, 14, 20), have applied the species name alone (*S. graminum*) to the fungus on wheat. The distinguishing features between these fungi are, according to Desmazieres, that in *S. graminum* the pycnidia (called perithecia by him) are smaller (invisible) and closer together than in *S. tritici* and the

pycnospores are narrower and slightly enlarged at one end. He gave the length of the pycnospores of the two species as practically the same and did not give the exact width in either case. Passerini (24) gave the first complete measurements of *S. graminum*, stating that the pycnospores are from 1 to  $1.25\mu$  in diameter. Cavara (5) gave 1.5 to  $2.0\mu$  as the width of the pycnospores of *S. tritici*. Heretofore the size of the pycnospore has been the only means of distinguishing between the two organisms. Cavara (5) suggested that *S. graminum* and *S. tritici* were merely specialized strains of the same organism. Mangin (19) did not find *S. tritici* attacking wheat. He concluded that *S. graminum* was the only organism affecting wheat in France and that the pycnospores were often  $2\mu$  wide. Prillieux (27) and Ferrais (13), however, report *S. tritici* in France and give the diameter of the pycnospores as ranging from 3 to  $5\mu$  and 2.5 to  $5\mu$  respectively. Voglino (36) stated that these two species of *Septoria* would be separated only by artificial culture work. Beach (1) considered that the organism found in Illinois was morphologically more like *S. tritici* Desm. than *S. graminum* Desm. and that since *S. tritici* Desm. was the first described he called the organism he found in Illinois *Septoria tritici* Desm. Numerous collections have been made from wheat fields in this vicinity at all seasons of the year for the past two years and specimens have been sent from Virginia, Indiana, North Carolina, Kentucky, Alabama, Illinois, Kansas, Nebraska, Minnesota, North Dakota, South Dakota, Oregon, and California, and in all of this infected material were found lesions comparable to those of the type material of *S. tritici* and pycnospores that measured more than  $1.5\mu$  in diameter. The pycnospores measured on an average  $2.5$  to  $3\mu$  in diameter with extremes at  $1.5\mu$  and  $4\mu$ .

Through the kindness of Dr. Etienne Foëx of Paris, portions of the type material of both *S. tritici* and *S. graminum* have been examined. The type, *Septoria graminum* Desm., is distinct from species collected on wheat near Madison, Wisconsin. In the type material the lesions on the leaves are long, narrow and decidedly limited by the veins. The pycnidia are indistinguishable to the unaided eye except by careful examination.

They are seriatly arranged parallel with the veins of the leaf. The pycnospores measured 1 to 1.5 by 30 to  $50\mu$ .

In contrast the *Septoria* lesions on wheat in this vicinity are circular to oblong, more or less limited by the veins. The pycnidia are readily seen without a lens and are more or less scattered. The pycnospores measure 1.75 to 3 by 40 to  $75\mu$ . By direct comparion it is evident that the *Septoria* organism on wheat in this vicinity is not *Septoria graminum* Desm.

On the other hand, type material of *S. tritici* Desm., furnished by Dr. Foëx compared very favorably with the organism found on wheat in this vicinity. The pycnosporos from the type material measure 2 to 3 by 40 to 75 $\mu$  and the pycnidia and lesions are very similar to those

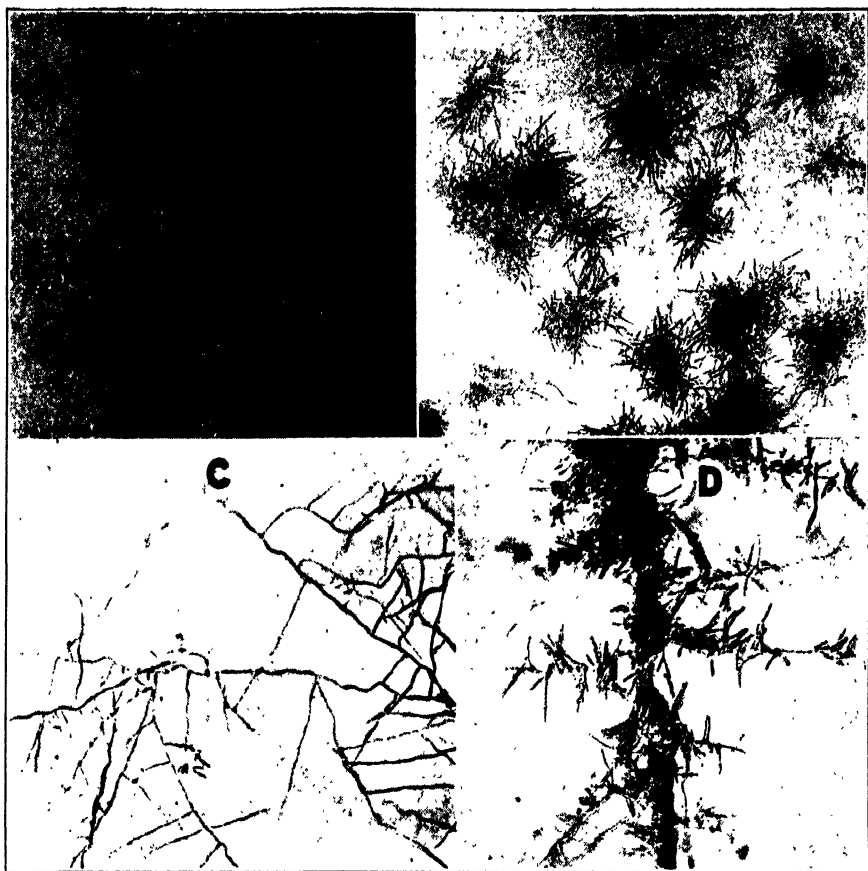


Fig. 11. Photomicrographs of *S. tritici* in artificial culture. A. Growth 24 hours after placing pycnosporos on potato-dextrose agar. B. Same 48 hours later. D. Hyaline hyphae bearing conidia. C. Olivaceous hyphae from same colony partly shown in D.

found on the Wisconsin material. The two undoubtedly belong to the same species.

It is observed from the original description of Desmazieres (10) that *Septoria graminum* Desm. was not described on wheat; this is evident from the fact that the type material does not appear to be on wheat nor does the original description so state. Furthermore, Desmazieres (11) had previously described *Septoria tritici* on wheat. It seems probable

that he (12) established the species *S. graminum* to include *Septorias* with pycnospores very similar to the type found on many wild grasses. He then apparently chose the name *graminum* for the more generalized usage, since it seemed more appropriate for this than *tritici*. He then listed *S. tritici* as *S. graminum* var. *b. tritici* signifying a variety specialized to wheat, and the form on oats as *S. graminum* var. *c. avenae* signifying a variety specialized to oats. Desmazieres (10, 11) did not distinguish the varieties *b. tritici* and *c. avenae* from the type *S. graminum* on pycnospore characters. He gives only the length of the pycnospores of var. *b. tritici* and states that the pycnospores of var. *c. avenae* are identical with the type. However, it is noted in the original description of *S. graminum* that the pycnospores are a little more slender than they are in *S. tritici*.

It is apparent from the evidence at hand, that in any case the proper name to apply to the form on wheat is *S. tritici* Desm. It was established first and was described on wheat. *Septoria graminum* was described later and was not described on wheat. Hence in this paper the writer accepts, for the form on wheat leaves, the name *Septoria tritici* Desm.

### Morphology

*Mycelium*.—In artificial culture media *S. tritici* develops two types of mycelium. One type is hyaline, thin walled, septate, branched, conidia bearing, 1.5 to 2 $\mu$  in diameter (Fig. 11 C). The other type is olivaceous, black in dense masses, walls about twice as thick as in the hyaline hyphae, branched, more closely septate, non-conidia bearing, and 2 to 2.5 $\mu$  in diameter (Fig. 11 D). The young hyphae are more or less homogeneous while cells of the older hyphae are more or less filled with vacuoles.

*Pycnidia*.—Pycnidia are subepidermal, in sub-stomatal chamber, globose to subglobose (Pl. XXXIII, B and Fig. 4), in rows parallel to the vascular strands (Pl. XXXV, A), brown to black, wall smooth, pseudo-parenchymatous, 1 to 3 cells thick, 80 to 150 $\mu$  in diameter. Ostiole circular to oval, slightly raised, 12 to 20 $\mu$  in diameter.

*Pycnospores*.—Pycnospores are slender, cylindrical, straight or slightly curved, hyaline, 3 to 7-septate, ends rounded, contents homogeneous or slightly guttulate, 1.75 to 2.7 by 39 to 70 $\mu$ , averaging 2.2 by 50 $\mu$  for the summer spores, and 2.5 to 3.5 by 52 to 85 $\mu$  averaging 3 by 76 $\mu$  for the winter spores.

As noted pycnospores collected in winter have been found to be considerably larger than those collected in summer. Beach (1) was the first to record this. The writer has found that in winter the pycnospores average 1 to 1.5 $\mu$  wider and 15 to 20 $\mu$  longer than in summer.

In the greenhouse several pots of seedlings of *Triticum aestivum* (Marquis) were inoculated with pycnosporos of *S. tritici* Desm. collected on winter wheat leaves in February. These pycnosporos were distinctly longer and slightly thicker than pycnosporos collected on wheat in July. Other plants were inoculated with the shorter pycnosporos collected in July. Typical symptoms developed after eight or nine days and pycnidia were found on the blotches on the leaves after two weeks.

The symptoms developed on the two series of inoculated plants were similar. The lesions produced and the pycnidia formed were typical in both cases. Pycnidia and pycnosporos were examined under the microscope and in both cases they were alike. The pycnospore measurements were within the limits given for summer spores and in no case were pycnosporos found that compared in length with the pycnosporos collected in February. These experiments confirm results obtained by Beach (1) who conducted similar experiments. It appears from these experiments, which were repeated four times, that the pycnosporos of *S. tritici* Desm. are variable as to measurements at different seasons of the year. Table 2 shows the results of these experiments.

TABLE 2

*Summary of inoculation experiments on Triticum aestivum showing type of pycnospore produced when "winter" and "summer" types of pycnosporos were used as inoculum.*

Date of inoculation	Number of leaves		Date of observation	Type of pycnospore	
	inoculated	diseased		used as inoculum	reproduced in new lesions
Feb. 10	24	18	Feb. 26	short	short
	25	20		long	short
	Controls	0		none	none
Feb. 20	20	20	Mar. 10	short	short
	14	12		long	short
	Controls	0		none	none
Feb. 27	18	16	Mar. 15	short	short
	23	20		long	short
	Controls	0		none	none
Mar. 4	10	10	Mar. 18	short	short
	15	14		long	short
	Controls	0		none	none

*Conidia*.—Conidia which resemble the pycnospores very closely have been noted on inoculated wheat leaves in the green house and in abundance on artificial culture media. From these two sources the conidia are almost indistinguishable. The conidia, first referred to by Janczew-

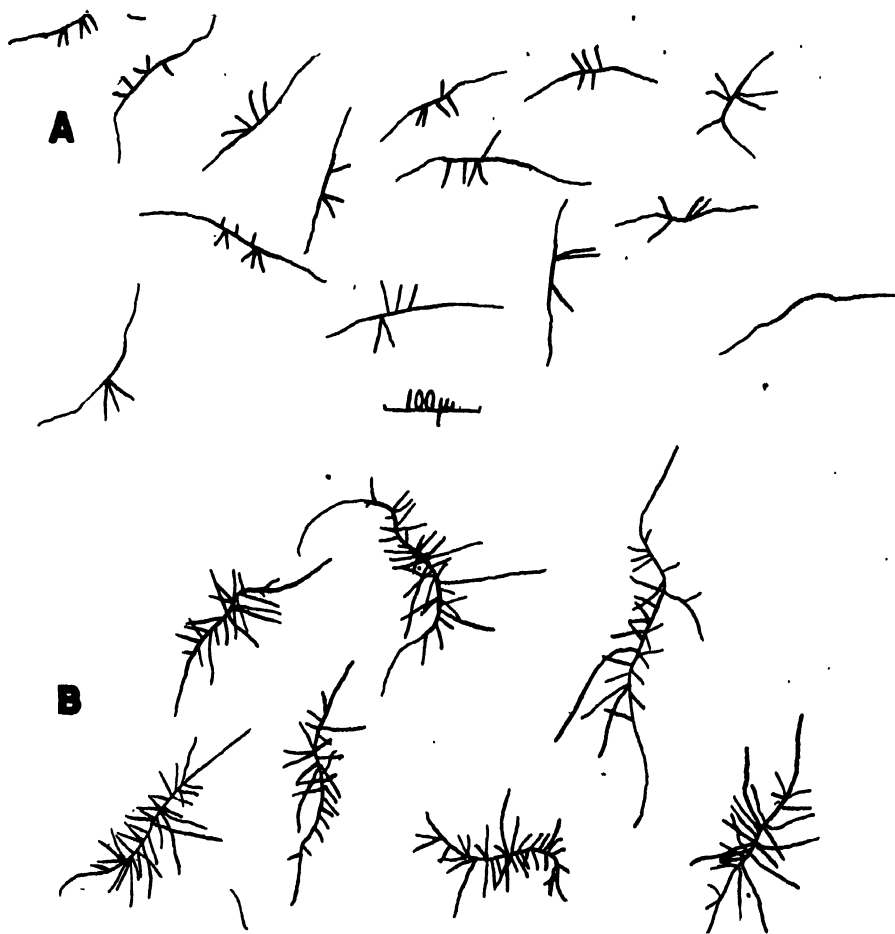


Fig. 12. Germinating pycnospores of *S. tritici*.

A. and B. Twenty-four and 48 hours growth respectively on potato-dextrose agar.

ski (17), are produced as secondary spores from the germinating pycnospores as shown in figure 14A and F. The conidia are cylindrical to fusiform, straight, or curved, ends rounded, hyaline, 3 to 7-septate, homogeneous or slightly guttulate to vacuolate, 2 to 3 by 35 to 65 $\mu$ . They are pathogenic in a manner parallel to the pycnospores.

*Perithecia*.—Not known.

## Physiology

*Cultural studies.*—*Septoria tritici* Desm. was secured in pure cultures on potato-dextrose agar by obtaining pycnospores from pycnidia collected on wheat leaves. The infected wheat leaves were soaked in water for a few minutes which caused the pycnospores to exude from the pycnidia. The spore suspension was then spread on the surface of slightly

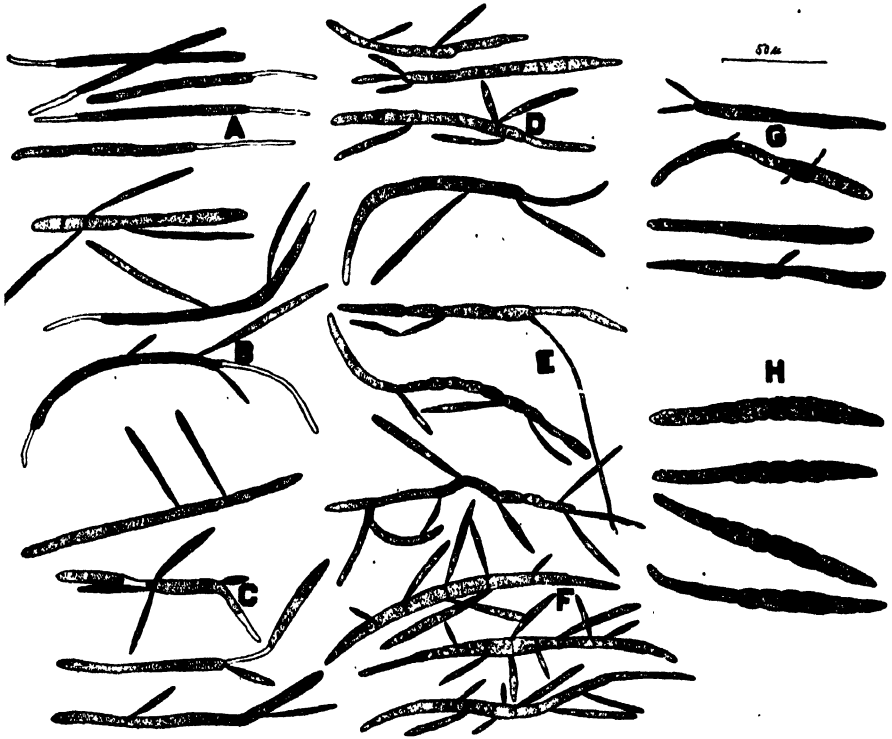


Fig. 13. Pycnospores of *Septoria tritici* suspended in water and incubated for 24 hours at different temperatures as follows; A. 2-3° C.; B. 7-8° C.; C. 10-12° C.; D. 14-16° C.; E. 18-20° C.; F. 22-24° C.; G. 28-30° C.; H. 32-36° C.

acidified potato-dextrose agar plates by means of a platinum loop. After incubation for 24 hours (Fig. 12 A), single, germinating spores were transferred to potato-dextrose agar in culture tubes. The colonies of conidia which developed for five days (Fig. 8 B) were pinkish or flesh colored, somewhat circular, convex, smooth, with a more or less irregular margin. This is in general agreement with conditions reported by Cromwell (7). After five to ten days mycelium was observed protruding from the margins of colonies. This mycelium was of two kinds (Fig. 11 C and D). One was straight, branched and hyaline, it produced lat-



eral conidia in great profusion but did not grow to any considerable length. The other developed at the same time or a few days later was somewhat coarser. The hyphae were undulating, branched, and of an olivaceous color. These hyphae grew rapidly and to considerable length, branched, and intertwined, forming a black mat of hyphae around the pink colonies. This dark colored mycelium did not produce conidia. After two weeks the surface of the colony began to turn dark colored and after four weeks it had a black stroma-like covering. Pycnospores were transferred to a number of different media and resulting growth showed strikingly different cultural characteristics. On potato-

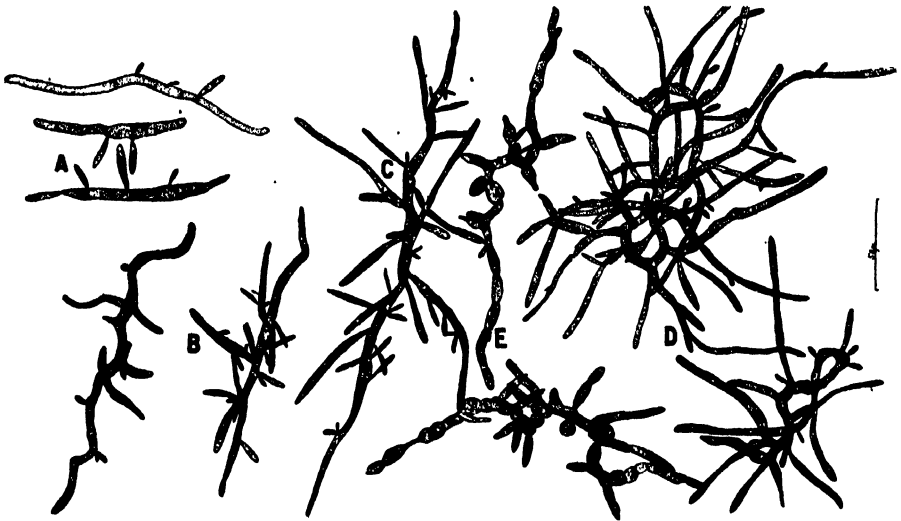


Fig. 14. Growth from pycnospores of *Septoria tritici* on potato-dextrose agar incubated 48 hours at different temperatures. A. 6-8° C.; B. 10-12° C.; C. 14-16° C.; D. 18-22° C.; E. 26-30° C.

dextrose agar the growth was very much as noted above except that after the first week the surface began to become wrinkled. The growth heaped up and formed folds (Fig. 15 A and B 3), later turning black. On nutrient agar no dark coloring took place, otherwise it developed very similar to the development on potato agar, but very much slower. On oatmeal agar the growth was similar to the growth on potato agar until the whole surface became black. White mycelium then began to develop and after four weeks it had completely covered the black surface of the culture. Transfers were made from the conidia and from the mycelium and after several successive transfers the cultures from conidia developed only conidia and those from mycelium developed only white mycelium. Pycnidia, containing pycnospores very similar

to those collected on wheat leaves, were developed on both potato and oatmeal agar cultures. Perithecia have not been found. Isolations from the large pycnosporos developed in winter and those from the smaller pycnosporos developed in summer are indistinguishable in culture.

*Relation of temperature to growth in culture.*—Pycnosporos from wheat leaves were isolated and transferred singly to each of a number of potato agar culture tubes and incubated at different temperatures for ten days. The greatest amount of growth took place at from 20 to 24° C. Above and below this temperature the amount of growth was less; there was no growth below 6° C. nor above 32° C.

*Relation of acidity and alkalinity to growth in culture.*—Several series of test tubes containing 10 cc. each of potato-dextrose agar were made up, their hydrogen-ion readings ranging from pH 2.5 to pH 9.2. Conidia grown in culture were transferred to these agar tubes and incubated for twelve days. It was found that vigorous growth took place on all cultures in each series with pH readings between 3.8 and 8. Below pH 3.8 and above pH 8 growth was retarded. There was considerable difference in the color of the organism in the different cultures. The organism growing at pH 2.5 was of a vivid pink color and the one grown at pH 9.2 almost white. Different shades developed between these extreme colors, depending on the hydrogen-ion concentration. A band of black hyphae developed (Fig. 15 A) around the margin of the mass of pink spores in each of the cultures except at a pH of 9.2. This band became wider and more dense in the cultures of the series up to pH 6.2 where the maximum amount of black hyphae was produced. Toward the alkaline end of the series this black color diminished and at pH 9.2 there were no black areas whatever in the culture and spore mass was white.

*Spore germination.*—Conidia and pycnosporos showed no difference in manner of germination. Conidia grown in culture behaved the same in germination as pycnosporos grown in culture. In water there was evidence of germination after four hours. On potato-dextrose agar the spores enlarged considerably and from three to seven septa were evident after about six or seven hours. From 1 to 4 buds appeared close together along the sides of the spore, usually near the septa (Fig. 11 A). These buds developed into conidia-like bodies as described by Jaczewski (17) and after from 24 to 30 hours germinated. In this way colonies of conidia developed as previously described. Very scant mycelium was found at this time (Fig. 11 B). The mass of conidia became dense and heaped up as shown in Figure 8 B.

*Relation of temperature to germination.*—Series of germination experiments of pycnospores from wheat leaves were conducted in the different chambers of a graduated incubator both on potato-dextrose agar and in hanging drops of water. In each case the cultures were incubated for 24 hours, when certain observations and camera lucida drawings were made (Figs. 13 and 14). In the water cultures at the lowest temperature (2 to 3° C.) a hyaline tube, which appeared to be empty, had developed. Some swelling had taken place but no septa were visible. At 7 to 8° C. and at 10 to 12° C. a few conidia developed, but their formation was not regular, empty cells being frequently found. Some septation had taken place. At from 14 to 24° C. germination was more uniform. The conidia appeared to be normal and the septa were regular. At from 28 to 34° C. extensive swelling occurred. There was also visible septation but little or no germination. Certain cells tended to become thick walled and chlamydospore-like, especially at the highest temperature (32 to 34° C.). The most favorable temperatures for germination in water were 22 to 24° C. Little or no germination took place at 2 to 3° C. or at 33 to 34° C. On the agar cultures the pycnospores germinated in much the same manner as they did in water, except that the germination seemed to be somewhat slower. The minimum, optimum, and maximum temperatures for germination were relatively the same in each case.

#### Pathogenicity

*S. tritici* is pathogenic on the leaves or leaf sheaths only of the host plants. Infected wheat plants have been collected during every month of the year. This disease was most destructive on seedlings of wheat upon which it has been found from the time they appear above the ground. (Pl. XXXIV, A). The fungus will develop in late fall or early spring, when temperature conditions are not favorable for the rapid development of the seedlings. At this time the seedlings are rapidly overcome by the fungus and very often killed. The organism was isolated from wheat leaves in the usual manner. Several days after the pycnospores were transferred to culture tubes the colonies had spread over the surface of the agar and ten days after the cultures were made conidia were formed. The conidia were suspended in distilled water and sprayed by means of an atomizer on the leaves of wheat seedlings. The seedlings were kept in a moist chamber for three days immediately following inoculation and then removed to a greenhouse bench. Eight or nine days after inoculation a yellow mottling began to appear on the leaves and after 13 to 14 days mature pycnidia had developed on the yellowed

areas which began to die and become brown. Pycnospores, which compared in every respect with the pycnospores originally used, were isolated from the artificially produced pycnidia as formerly described. They were carried in culture with a parallel series of cultures from the original material and no differences were noted.

*Cross inoculations with S. tritici.*—Beach (1) conducted a number of inoculation experiments in which he used several cereals and grasses as hosts. He reported negative results however for all plants tried except *Triticum vulgare*. The writer conducted inoculation experiments both in the greenhouse and in field plots on about forty species of host plants including the cereals and certain related grasses. The inoculum in the form of a pycnospore suspension was applied by atomizers. The inoculated plants were covered with paper bags for periods of from 48–72 hours depending on weather conditions. The bags were then removed. The results are given in table 3 and shown diagrammatically in figure 16.

TABLE 3

*Summary of results from inoculating various grains and grasses with Septoria tritici Desm.*

Hosts	Sources of inoculum <sup>1</sup>	No. of leaves		Hosts	Sources of inoculum <sup>1</sup>	No. of leaves	
		Inoculated	Dis-eased			Inoculated	Dis-eased
<i>Avena barbata</i>	1	153	0	<i>H. v. nigrum</i>	1	179	0
	2	21	0		2	34	0
	3	7	0		3	8	0
<i>A. brevis</i>	1	137	0	<i>H. v. pallidum</i>	1	188	0
	2	25	0		2	36	0
	3	6	0		3	15	0
<i>A. fatua</i>	1	133	0	<i>H. v. trifurcatum</i>	1	175	0
	2	32	0		2	28	0
	3	5	0		3	7	0
<i>A. nuda chinensis</i>	1	152	0	<i>Triticum aestivum</i> (Marquis)	1	156	122
	2	26	0		2	33	17
	3	7	0		3	15	9
<i>A. sativa</i> (C. I. 1375)	1	31	0	<i>T. a. lutescens</i> (Harvest Queen)	1	158	121
	2	14	0		2	35	8
	3	0	0		3	13	4

<sup>1</sup> The sources of inoculum are as follows: 1. Pycnospores from wheat species. 2. Pycnospores from rye. 3. Pycnospores from *Poa pratensis*.

Hosts	Sources of in- oculum	No. of leaves		Hosts	Sources of in- oculum	No. of leaves	
		Inoc- ulated	Dis- eased			Inoc- ulated	Dis- eased
A. sativa (Culbertson)	1	28	0	T. a. miltura (Red Wave)	1	136	103
	2	131	0		2	23	13
	3	9	0		3	9	7
A. sativa nigra	1	126	0	T. compactum	1	149	82
	2	39	0		2	36	17
	3	19	0		3	6	1
A. sativa orientalis	1	137	0	T. dicoccum barrum	1	142	95
	2	19	0		2	24	11
	3	11	0		3	5	3
A. sterilis (a)	1	118	0	T. dicoccum atratum	1	197	139
	2	28	0		2	32	12
	3	11	0		3	11	4
A. sterilis (low belt)	1	12	0	T. durum	1	140	102
	2	6	0		2	26	13
	3	7	0		3	15	9
A. sterilis (Rust proof)	1	18	0	T. monococcum	1	154	105
	2	11	0		2	44	22
	3	0	0		3	11	7
A. sterilis (b)	1	173	0	T. polonicum	1	160	108
	2	22	0		2	31	11
	3	10	0		3	12	5
A. strigosa	1	152	0	T. spelta	1	186	129
	2	22	0		2	31	10
	3	9	0		3	21	10
Hordeum deficiens	1	149	0	T. turgidum	1	148	115
	2	29	0		2	28	14
	3	10	0		3	7	4
H. horsfordianum (Oregon)	1	161	0	Secale cereale	1	144	95
	2	30	0		2	49	40
	3	6	0		3	23	3
H. horsfordianum (S. Dak.)	1	167	0	Agropyron repens	1	166	0
	2	34	0		2	33	0
	3	8	0		3	17	0

Hosts	Sources of in- oculum <sup>1</sup>	No. of leaves		Hosts	Sources of in- oculum	No. of leaves	
		Inoc- ulated	Dis- eased			Inoc- ulated	Dis- eased
<i>H. jubatum</i>	1	79	0	<i>Agrostis platonifera</i>	1	14	0
	2	0	0		2	0	0
	3	12	0		3	0	0
<i>H. distichon erectum</i>	1	173	0	<i>Andropogon nirtus</i>	1	10	0
	2	31	0		2	0	0
	3	11	0		3	0	0
<i>H. distichon nudum</i>	1	165	0	<i>Andropogon sorghum</i>	1	14	0
	2	26	0		2	0	0
	3	9	0		3	0	0
<i>H. distichon nutans</i>	1	164	0	<i>Arundo arenaria</i>	1	9	0
	2	23	0		2	0	0
	3	6	0		3	0	0
<i>H. vulgare coerulescens</i>	1	166	0	<i>Astrebula triticoides</i>	1	15	0
	2	28	0		2	0	0
	3	10	0		3	0	0
<i>H. v. hexa- strichum</i>	1	152	0	<i>Avia flexuosa</i>	1	7	0
	2	27	0		2	0	0
	3	7	0		3	0	0
<i>H. v. himalaya</i>	1	147	0	<i>Bromus inermis</i>	1	91	0
	2	24	0		2	11	0
	3	13	0		3	7	0
<i>Chaetochloa italica</i>	1	124	0	<i>Microlaena stipoides</i>	1	16	0
	2	17	0		2	0	0
	3	7	0		3	0	0
<i>Chaetochloa viridis</i>	1	19	0	<i>Panicum brizanthum</i>	1	17	0
	2	0	0		2	0	0
	3	0	0		3	0	0
<i>Cynosorus cristatus</i>	1	12	0	<i>Panicum clandestinum</i>	1	98	0
	2	0	0		2	23	0
	3	0	0		3	6	0
<i>Echinochloa crus-galli</i>	1	153	0	<i>Panicum floridum</i>	1	7	0
	2	22	0		2	0	0
	3	3	0		3	0	0

Hosts	Sources of inoculum	No. of leaves		Hosts	Sources of inoculum	No. of leaves	
		Inoculated	Diseased			Inoculated	Diseased
Eschlaena luxurians	1	14	0	Pennisetum glaucum	1	125	0
	2	0	0		2	17	0
	3	0	0		3	9	0
Festuca arundinacea	1	11	0	Poa pratensis	1	139	52
	2	0	0		2	31	12
	3	0	0		3	13	11
Holcus halepensis	1	14	0	Poa trivialis	1	8	0
	2	0	0		2	0	0
	3	0	0		3	0	0
Lolium perenne	1	9	0				
	2	0	0				
	3	0	0				

Table 3 and figure 16 show that *Septoria tritici* is confined to the wheat species, rye, and *Poa pratensis*. This disease has been reproduced on these hosts consistently and has never been found on any of the other inoculated grains or grasses.

During October, 1920, several plants of each of 250 winter wheat varieties were inoculated with a pycnospore suspension of *Septoria tritici*. The inoculated wheat seedlings were covered with petri dish covers for 48 hours immediately after inoculation. Approximately two weeks after inoculation data were taken which showed 100 per cent infection in all varieties with the exception of five which had been destroyed by rodents.

#### CAUSAL ORGANISM IN RELATION TO THE PRODUCTION OF THE DISEASE

##### Seasonal development

During every month of the past two years viable pycnospores have been collected in the field in the vicinity of Madison, Wisconsin. There has always been an abundance of pycnidia on dead wheat leaves during the winter months. Early in spring when the winter wheat plants resume growth, the pycnospores which exude from the pycnidia as a result of the early rains germinate. Following infection irregular blotches appear on the wheat leaves. In these irregular, light colored blotches black, speck-like pycnidia form. As the wheat plants develop the

newly unrolled leaves become infected. In favorable springs, that is when there are abundant warm rains and foggy and cloudy days, the



Fig. 15. A. Growth of *Septoria tritici* on potato-dextrose agar adjusted to different degrees of acidity and alkalinity from pH 2.5 to pH 9.2. Note blackening around organism at all except pH 9.2 (extreme right). B. Growth of *S. tritici* on different artificial culture media. Cultures incubated at room temperature for 28 days (removed from culture tubes). (1) On oatmeal agar; (2) On nutrient beef broth agar; (3) On potato-dextrose agar.

fungus invades the whole plant and sometimes kills it. When the culms lengthen infection usually decreases. However, in certain ex-



perimental plots of Blue Stem wheat during June, 1921, at Madison, Wisconsin, the spread of the disease on the plants was not lessened and at blossoming time every leaf including the flag leaf was entirely killed. This was possibly an exceptionally heavily diseased plot but nevertheless it showed how completely plants might become invaded. After the flowering stage very little new infection took place until volunteer seedlings began to appear in August and September. These seedlings became heavily infected but they were seldom killed by the disease because of their rapid growth. These volunteer seedlings which become infected from the old infections on old straw and stubble are very important in the spread of the disease. They serve to carry over the disease from harvest to the time the fall plantings of winter wheat are up. The seedlings of the fall plantings are usually sufficiently developed by October 1 to become infected and the source of infection is largely from volunteer seedlings. During late fall when the plants grow more slowly the fungus spreads to all parts of them and in certain experimental plots serious losses were observed due to this disease. Early fall plantings were injured to a less extent than late October and November plantings. Some loss was observed, however, on the early planted grain. In certain plots as many as 5 per cent of the tillers were killed but a whole plant was never found completely destroyed by the fungus. In late plantings small plants may be killed by the attack of the fungus; during the winter months the pycnosporos retain their viability but the lesions do not enlarge.

#### Mode of Overwintering, Viability and Longevity of Pycnosporos

As previously stated, the fungus has been collected during the winter months of two succeeding years and the pycnosporos were found to be viable throughout the winter. A number of heavily infected wheat leaves were collected in July, 1920, and placed out of doors on the ground. During the winter of 1920 and 1921 infected leaves were taken to the laboratory and the pycnosporos tested for viability. At no time during the winter did the percentage of germination fall below 86 per cent. The pycnosporos, when retained in the pycnidia, survived the winter very readily.

The viability of the pycnosporos was not materially changed during the fall, winter and spring months, but beginning with the summer months, when the pycnosporos became a year old, some decrease in viability was observed. This decrease was not caused by the warm dry weather alone, because pycnosporos collected in spring and kept during the summer showed no decline in viability. It was, therefore,

concluded that the age of the spore was an important factor in viability and that its decrease was due to age and warm dry weather together. Pycnospores kept in the laboratory in a pasteboard box showed a slight decrease in viability after six months. At the end of the year the per-

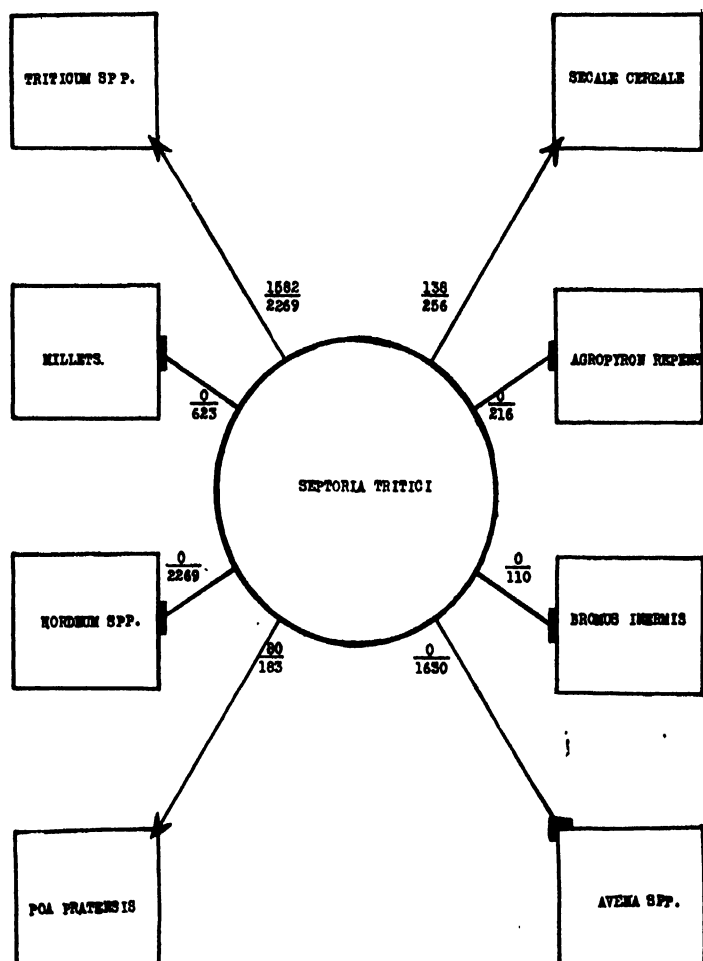


Fig. 16. Diagram showing results given in table 3. The source of inoculum is shown in the circle, the hosts inoculated in the rectangles. The denominator of the fraction designates the number of leaves inoculated and the numerator the number of leaves diseased at the end of 15 days. Arrows indicate infection.

centage of viable spores had decreased 50 per cent, and at the end of eighteen months to from 30 to 35 per cent. Sixty per cent of the pycnospores kept out of doors germinated after eighteen months.

### Modes of Dissemination

On November 1, 1920, the dead plants of winter wheat heavily infected with *Septoria tritici* and thickly studded with pycnidia, were tied into small bundles with a cord. The leaves were left attached to the plant and the cord was tied to a firmly driven stake. On April 1, 1921, the bundles were collected. Instead of finding the leaves as they were left in the fall only short pieces were found which had been protected by the knot in the string and at which place the leaves had resisted disintegration by being closely packed together (Fig. 9 B). The parts of the leaves not held by the string had entirely disintegrated and were disseminated during the period between November 1, 1920, and April 1, 1921. The wind was probably the greatest agent of dissemination. It carries disintegrated leaf tissues containing pycnidia and the free pycnidia themselves. The wind, however, is probably not as important as rain in scattering the pycnosporos. The wind may scatter the pycnidia over long distances and the rain by splashing the pycnosporos enlarges the range of infection from a single pycnidium. The pycnosporos become very evenly distributed during rainy days. The writer has observed that following such rains the prostrate leaves became more heavily infected than those that remain erect.

### Time and Mode of Natural Infection

In the vicinity of Madison, Wisconsin, wheat plants have become infected during every month of the year except January and February. During the late fall and early spring the amount of new infection was less than during the late spring, summer and early fall.

The mode of infection was studied on leaves of wheat inoculated with pycnosporos in the greenhouse. The leaves were sprayed with a water suspension of pycnosporos of *S. tritici* by means of an atomizer. The plants were placed in a moist chamber immediately after inoculation and left there for 48 hours. They were then placed on a bench in a greenhouse kept at a temperature from 65 to 75° F. Every twelve hours for a period of six days after inoculation, pieces of inoculated leaves were placed in a solution of equal parts of 95 per cent alcohol and glacial acetic acid. They were left in this solution for at least 24 hours during which time the solution was changed once or twice. As a result the cells were killed with very little plasmolysis and the chlorophyll was removed. The pieces of leaf were white after 24 hours. They were then submerged for 15 or 20 minutes in Pianezze IIIb stain as used by Vaughan (35). The pieces were then washed in water and examined under the microscope. The pycnosporos and mycelium were stained

a dark blue and the host tissue was slightly tinged red. By examination in this way the extent of growth and place of penetration were readily seen. After penetration had taken place the method of staining the leaf pieces was changed somewhat. The stain was diluted with three to five parts of 25 per cent alcohol, and the pieces of leaves were left in the stain for from 18 to 24 hours. This stained the whole pieces dark blue. They were then washed in water, passed through 95 and 100 per cent alcohol respectively and then put in carbol-turpentine to clear. When cleared the leaf pieces were passed through xylol and mounted in Canada balsam. The leaf tissue was slightly bluish-green while the fungus was a deep rose. The fungus was most easily detected on the surface of the leaf after it was washed in water and before it was placed in alcohol. When placed in alcohol the blue color of the fungus disappeared leaving it rose-red. Examination of a large number of pieces of leaf tissue prepared in this way showed that the germ tubes penetrated the cuticle at a point directly above adjacent walls of the epidermal cells. They grew down between the epidermal cells into the intercellular spaces. There was some evidence of stomatal infection but such instances were very rare and mycelium entering the stomata were not found directly connected to pycnospores of *Septoria*.

#### Period of Incubation

The incubation period varied from eleven to fifteen days depending upon environmental conditions. After inoculation no change in the host was observed from the normal until the sixth or seventh day. Yellowish blotches began to appear and after eight days the inoculated leaves became mottled. On the tenth day light brown specks more or less scattered over the light yellow blotch were observed by the aid of a hand lens. These specks, the pycnidia, darkened and finally became black. During May and June the period of incubation was the shortest observed, being only eleven days. The longest periods required for the reproduction of the disease was during the late fall when the temperature was comparatively low.

#### Pathological Anatomy

The study of the pathological anatomy of the disease was made possible by the use of Planeze IIIb stain and methods already described. At the time the first symptoms appeared on the leaves it was found that the mycelium of the fungus had invaded a large portion of the light colored blotch. After penetration and growth down between the epidermal cells the hyphae branched and single strands often grew parallel

with the epidermis directly under it (Pl. XXXVI, A and B), in various directions but most commonly lengthwise of the leaf. The side branches followed very closely the wall of the cells that were adjacent to the epidermal cells. The septations and vacuoles in the hyphae were easily seen. Occasionally there was a web of closely interwoven hyphae in the substomatal chamber. At this time these webs were found to be made up either of one or two strands of hyphae which had branched once or twice, or of one or two closely intertwined balls of hyphae situated directly under each end of the stomata in the substomatal chambers (Pl. XXXVI, B and C). The hyphal strands that grew deeper into the host tissue were rather irregular as to the direction of growth but they were always found in the intercellular spaces. It was observed that the hyphae grew through the tissue to the lower epidermis. Here they turned and grew parallel to the epidermis or turned back again and grew deeper into the tissue. No haustoria were observed. The spread of the fungus in the host tissue was more or less limited by the vascular bundles (Fig. 12 A). The hyphae appeared to be confined to the host tissue between the bundles except where the hyphae were very numerous, in which case certain of the bundles were killed. Under such conditions the hyphae were observed to grow across vascular bundles growing directly under the epidermis. When they were past the vascular bundle they branched profusely again and grew in all directions. Further examination of the diseased leaf tissues made at the time the pycnidia were mature showed that the pycnidia always occupied the substomatal chamber, so completely filling it as to occasionally crowd the parenchymatous cells that bordered on the substomatal chamber out of position. The pycnidia were more or less flattened on the top next to the epidermis. The ostiole which in culture was circular to oval conformed to the shape of the stomata and was without exception directly under it. The small oval to lenticular shaped cells forming the pycnidial wall were compact. The outer cells were covered with mycelial strands connecting with the hyphae in the adjacent host cells. These host cells, a dozen or more in number, adjacent to the pycnidium, which were dead and in most cases collapsed and irregular in form, were penetrated by the hyphae. Adjacent to the dead areas host cells have been found in various transition stages from dead to healthy.

#### SUMMARY

1. Speckled leaf blotch of wheat caused by *Septoria tritici* Desm. is widely distributed in the wheat growing regions of the world. The disease also occurs on rye.

2. The disease is of economic importance, locally, during recent years especially on wheat.

3. The disease occurs only on the leaves of the host plants.

4. The ascigerous stage of the fungus is not definitely known.

5. The mycelium of the fungus is composed of branched, hyaline, septate hyphae. The pycnidia are subepidermal, subglobose, smooth, black. The pycnosporos are cylindrical, septate, hyaline, 1.7 to 3.4 by 39 to 86 $\mu$ . During the summer the pycnosporos average 2.2 by 55 $\mu$  and during winter 3 by 76 $\mu$ .

6. The fungus grows well on slightly acid, potato-dextrose agar, producing mycelium, conidia and pycnidia.

7. Cardinal temperatures for spore germination are as follows: minimum 2 to 3° C., optimum 22 to 26° C., and maximum 32° C.

8. Cross inoculation experiments show *S. tritici* to be pathogenic on wheat species, rye and *Poa pratensis*.

9. The fungus overwinters in the pycnidial stage, the pycnosporos remaining viable if retained in the pycnidia.

10. Infection takes place by direct penetration of the cuticle. The germ tube then grows between the epidermal cells and develops further between the cells of the parenchyma. Host cells are not penetrated until after they are killed.

11. The period of incubation is from 11 to 15 days.

12. The mycelium is intercellular, the pycnidia are subepidermal, in the substomatal chambers. The pycnosporos escape through the ostiole.

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GLUME BLOTCH OF WHEAT

A. Photomicrograph of a pycnidium of *S. nodorum* Berk. in cross section and a perithecium (below).

B. Photomicrograph of cross section of a pycnidium of *S. nodorum* Berk. C. Culture of *S. nodorum* Berk. showing colonies of conidia intermixed with the white mycelium.

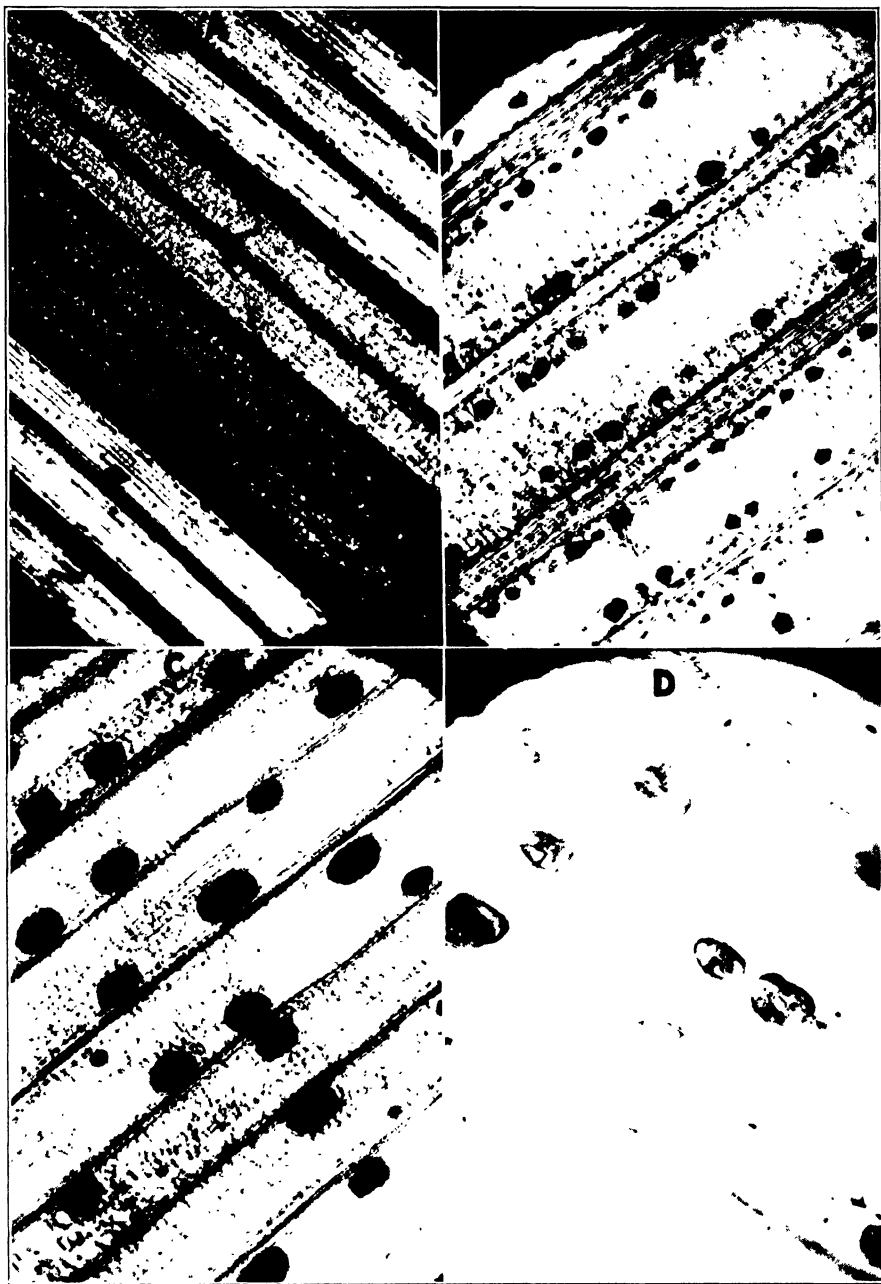




## SPECKLED LEAF BLOTCH OF WHEAT

A. Tillers of winter wheat heavily infected. Seeds sown in August, 1920. Plants collected April 1, 1921. B. Seedlings of winter wheat heavily infected. Seeds sown in November, 1920. Plants collected April 1, 1921.

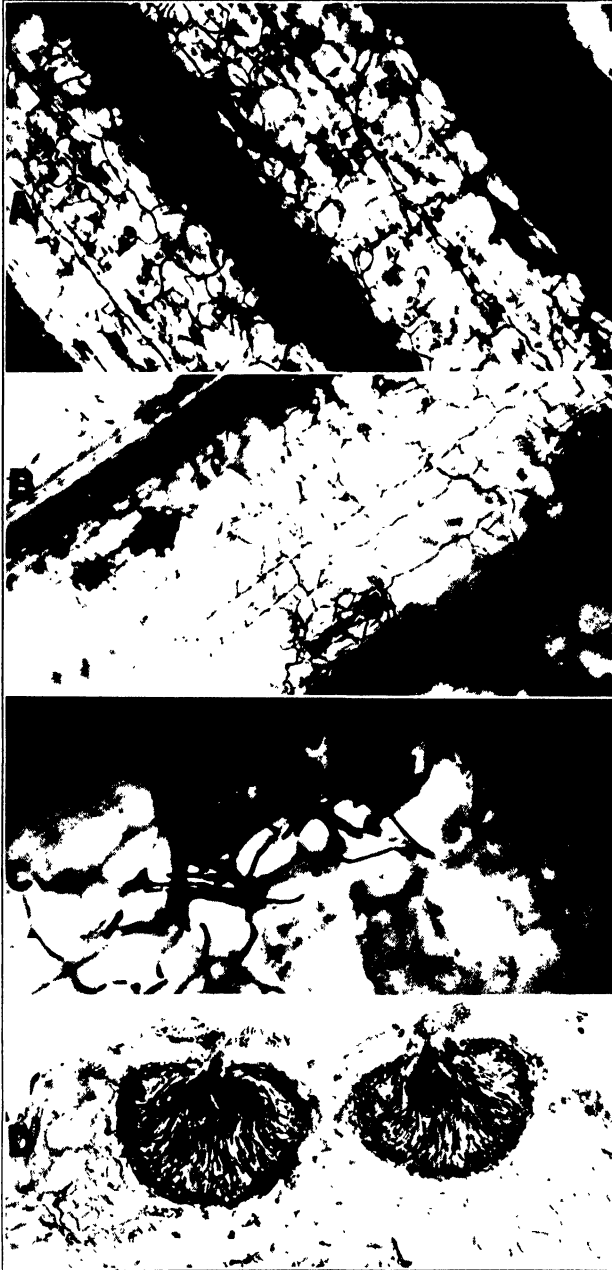




## SPECKLED LEAF BLOTCH OF WHEAT

Photomicrographs of portions of wheat leaves infected with *S. tritici*. A. Showing tendency toward limitation of spread of infection by veins. 1, Uninvaded; 2, heavily invaded; 3, lightly invaded. B. Leaf sheath showing seriate arrangement of pycnidia. C. Leaf blade showing variations in shape and size of pycnidia. D. Showing pycnidia, ostiole and escaping pycnosporangia.





## SPECKLED LEAF BLOTCH OF WHEAT

Photomicrographs showing hyphae and pycnidia of *S. tritici* in wheat leaves. A. Showing the hyphae among the parenchyma cells. B. Same as A, showing beginnings of formation of pycnidia below stomata. C. Aggregations of hyphae under a stoma—an early stage in the formation of a pycnidium. D. Cross section of two mature pycnidia imbedded in dead, shrunken host tissue.





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## ERRATA

Page 165, line 1 of legend should read "Fig. 2. *Macrosporium carotae*. A. Stem of a mature carrot plant, etc."

Page 279, line 34, "not likely" should be "unlikely."

Page 281, line 13, "Bordeaux Burgundy mixture" should be "Bordeaux or Burgundy mixture."

Page 381, title heading "Roselinia" should be "Rosellinia"

Page 384, line 14, "Roselinia" should be "Rosellinia."

Page 385, page heading, "Roselinia" should be "Rosellinia."

Page 390, line 15 "sore" should be "spore."

Page 396, line 18, "citullina" should be "citrullina."

Page 397, line 11, "Masse" should be "Massec."

Page 434, line 22, "Edgertown" should be "Edgerton."

Page 499, footnote belongs to bottom of page 497.





